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TROPHIC AND REPRODUCTIVE ECOLOGY OF A NEOTROPICAL CHARACID FISH *HEMIBRYCON BREVISPINI* (TELEOSTEI: CHARACIFORMES)

Ecología trófica y reproductiva del pez carácido neotropical *Hemibrycon brevispini* (Teleostei: Characiformes)

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ABSTRACT

Hemibrycon brevispini is a Neotropical characid fish endemic in La Venada Creek, a headwater tributary of the Quindío River of Colombia (Cauca River drainage). It is mainly a diurnal insectivore with a diet dominated by benthic dipterans (Chironomidae, Simuliidae, Psychodidae, Culicidae, Calliphoridae, Dixidae and Muscidae), hymenopterans (Formicidae and Vespidae) and ephemeropterans (Baetidae), as well as allochthonous prey and items eaten accidentally. Microhabitats of mountain streams with lower water velocity tend to have more riparian vegetation and the associated terrestrial arthropods that are consumed by *H. brevispini.* It has three peaks in reproduction: December, April and August. Average fecundity was 776 mature oocytes per female.

Key words. Diet, reproduction, conservation, Neotropical fishes, natural history.

RESUMEN

Hemibrycon brevispini es un pez carácido neotropical endémico de la quebrada La Venada, un afluente del río Quindío en Colombia (cuenca del río Cauca). Esta especie es predominantemente insectívora diurna, con una dieta dominada por dípteros (Chironomidae, Simuliidae, Psychodidae, Culicidae, Calliphoridae, Dixidae y Muscidae), himenópteros (Formicidae y Vespidae) y efemerópteros (Baetidae), además de presas de origen alóctono y otras definidas como consumo accidental. Los hábitats con baja velocidad de agua sustentan mayor vegetación ribereña asociada a artrópodos terrestres, consumidos por *H. brevispini*. Su reproducción tiene tres picos: diciembre, abril y agosto. Su fecundidad promedio es de 776 oocitos por hembra.

Palabras clave. Dieta, reproducción, conservación, peces neotropicales, historia natural.

INTRODUCTION

The genus *Hemibrycon* consists of fishes characterized by the presence of more than four teeth on the maxilla (in adults) (Eigenmann 1927, Román-Valencia *et al.* 2013). A phylogenetic analysis of *Hemibrycon* determined its monophyly based on four synapomorphies: ectopterygoids with widened ventral anterior projection, four to six times wider than posterior part; a red spot present in life on ventral margin of caudal peduncle; a postero-ventral projection on the pterotic and first infraorbitals gradually decreasing in width from posterior tip and located near posterior part of antorbital (Arcila-Mesa 2008). *Hemibrycon brevispini* Román-Valencia & Arcila-Mesa was described from La Venada and Quebrada Negra Creeks tributaries of the Santo Domingo River, Quindío River basin, upper Cauca, Andes of Colombia (Román-Valencia & Arcila-Mesa 2009).

Fourteen species of *Hemibrycon* have been described from the Cauca-Magdalena River Basin in Colombia, but there are few studies of their ecology that provide baseline information to determine their conservation status or provide guidelines for the management of many species that have relatively small geographical ranges and populations. In fact, habitat, extensive diet and reproductive data are only available for two *Hemibrycon* species from the Magdalena-Cauca River Basin: *H. boquiae* (Román-Valencia *et al*. 2008) and *H. quindos* (Román-Valencia & Botero 2006), but short notes have been published about of *H. brevispini* (Román-Valencia & Arcila-Mesa 2009), *H. antioquiae*, *H. fasciatus* and *H. cardalensis* (Román-Valencia *et al.* 2013), *H. cairoense* (Román-Valencia & Arcila-Mesa 2009), *H. paez*, *H. raqueliae*, *H. virolinica* and *H. yacopiae* (Román-Valencia & Arcila-Mesa 2010), *H. palomae* (Román-Valencia *et al.* 2010) and *H rafaelense* (Román-Valencia & Arcila-Mesa 2008). *H. brevispini* is endemic to La Venada and La Negra Creeks that are both tributaries of the Quindío River, in the upper Cauca River drainage (Román-Valencia & Arcila-Mesa 2009). Aspects of *H. brevispini* diet, reproduction and habitat were analyzed order to provide baseline information useful for conservation and management efforts of this endemic species and its habitat, especially considering the increasing impacts of hydropower and mining development in the Colombian Andes.

MATERIALS AND METHODS

Data collection and study area description. Sampling sites are distributed along the entire length of La Venada Creek, from its origin to its mouth where it discharges in to Quebrada Negra Creek. Fishes were collected in the middle and lower reaches of La Venada Creek (4° 26' 47.4" N & 75 ° 40' 44.3" W, 1661 m.a.s.l. and 4° 26' 54.9" N & 75° 40' 48.8" W, 1307 m.a.s.l.), a tributary of Quebrada La Negra Creek, which in turn is a tributary of the Santo Domingo/Quindío/upper Cauca River system in the Andes of Colombia. Thus, La Venada Creek is a primary or secondary stream, in the hierarchical classification system of Allan (1995). Fish were captured on two days of each month from July 2011 to November 2012, using a 2 x 0.5 m seine net, with 5 mm mesh and a 2 m cast net with a 10 mm mesh between 0900 and 1300 hr, sampling much of the creek. This period included dry seasons (June-August and January-February) and wet seasons (March-May and September-December) (Fig. 1).

A total of 122 specimens of *H. brevispini* were collected and placed on ice to decrease the rate of enzymatic digestion of stomach contents, as recommended by Bowen (1996). Samples were dissected the same day as collection at the Ichthyology Laboratory of the Universidad of Quindío, Armenia, Colombia (IUQ). An incision was made along the ventrum, and the stomach, intestine and gonads were extracted. After dissection, specimens were fixed in formalin (10%) for 15 days and then in 70% ethanol and deposited in the fish collection (IUQ). Gonads were weighed using an analytical balance (Adventurer-Ohaus H226) with 0.0001 g precision and subsequently preserved in 70% ethanol. Mitutoyo digital calipers with 0.01 mm precision were used to measure standard and total length of the fish, stomach length, stomach width and intestine length.

Habitat measurements. A pH meter (Hanna HI 921ON) was used to measure pH, air temperature and water temperature. A digital oxygen meter (OX1196) was used to measure dissolved oxygen and saturation. Geographical coordinates were recorded from a GPS unit (Garmin eTrex 10).

Diet. Prey items in fish stomach contents were identified to the lowest taxonomic resolution possible (order, family, genus) (Borror *et al.* 1992, Roldàn 1996). Stomach contents were analyzed using numeric and frequency methods (Hyslop 1980, Hynes 1950) and a volumetric method (Capitoli, 1992, Pedley & Jones 1978). The Index of Relative Importance (IRI) (Oda & Parrish, 1981), proposed by Pinkas *et al.* (1971) was used to determine the importance of each food item.

$$\boldsymbol{IRI} = \%Fo\ (\%N + \%V)$$

Where, %V= percent volume, % Fo= observed frequency percent and %N= proportion of food type.

Prey item identification was done for individuals in different states of digestion or based on diagnostic structures, however unidentified remains or parts of organisms were not treated as items; these are listed, but not included in the statistical diet analyses. The emptiness coefficient (*V*) (Hyslop 1980) was also calculated to reveal the months included

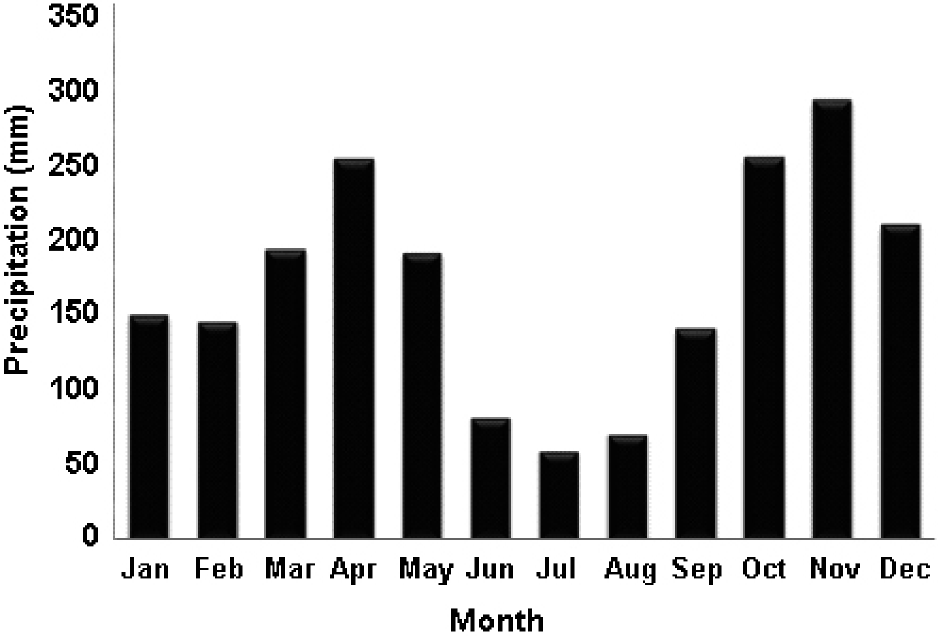

Figure 1. Multi-year monthly rainfall 1998-2005, station La Bella, Calarcá, Quindio, Colombia. Data of Cenicafé.

on the feeding period of the species,

$$V = \frac{n}{N} \times 100$$

Where, n= number of empty stomachs, N= total number of stomachs examined.

A centered principal component analysis was made using stomach contents abundances (%N) and the proportion of the total volume of each prey item (called %PCA after Billy *et al.* [2000]). In this method, total row (of diet items encountered in an individual stomach) is equal to 1. For the analysis, the families were grouped within orders to enable a better explanation of the diet. This analysis reveals the pattern of dispersion of prey found in stomachs. Representation on two axes (Gabriel 1981) was done with the first factorial plane; the position of each item is equivalent to the position of a stomach containing 100% of the prey species. Each stomach is at the centroid of the prey items, with each prey species being given a weight equal to its proportion in the stomach. Computations and graphical displays were performed with the ADE-4 package (Thioulouse *et al.* 1997) running in R-software (R Development Core Team 2013). Correlation analysis between the %PCA scores and standard length was performed to examine patterns of prey consumption among fishes of different sizes.

Abundance distribution normality of food items was evaluated using the Kolmogorov-Smirnov test with a 5% of significance (α= 0.05). Based on this a Kruskal-Wallis was done to evaluate differences in prey abundance (numbers of individuals) among seasons, sex and maturation stage. A correlation analysis based on log-transformed data was applied to examine relationships among the variables: standard length (SL), total length (TL), intestine length (IL) it is in mm, stomach width (STW) in g, stomach length (STL) in mm, stomach weight (STWE) in g, gonad weight (GW) in g, fecundity (FE) in number of oocytes and total weight (TWE) in g. The Past 2.11 (Hammer *et al.* 2001) and R-software (R Development Core Team 2013) programs were used for statistical analyses.

ANOSIM was used to test dietary preferences among food items. The similarity matrix was generated with the transformed data (log[x+1]) for consumed prey using the Bray-Curtis similarity and the observed relationships were compared based on 9999 permutations. The value of R lies between -1 and 1, where 0 indicates that low and high similarities are perfectly mixed, thus there is no preference in dietary items.

Condition factor (K). A condition factor (K) was calculated and used to evaluate the population's condition (Wootton 1992, Vazzoler 1996, Bagenal & Tesch 1978),

$$K = \frac{Wt}{Ls^{b}} \times 100$$

Where, Wt (g) = total weight, Ls (mm) = standard length and b, la relation length weight.

Reproduction. To determine the reproductive season, temporal variation in the gonadosomatic index (GSI) was evaluated. GSI was equal to

$$GSI = \frac{Wo}{Wc} \times 100$$

where Wc= Wt-Wo, and Wo (g) = gonad weight, Wt (g) = total weight, and Wc (g) = body weight (Vazzoler 1996). Spawning seasons were identified as peaks in mean GSI. Size at sexual maturity was determined using the graphic method of Sokal & Rohlf (1995) that identifies the maturation size as that for which 50% of the population is reproducing. Sex ratio was evaluated using chi-squared (X^2) and proportion of males and females.

Fecundity. Fecundity was determined using the dry subsample method (Ricker, 1971), and absolute fecundity (*Fa*) was calculated using only mature females according to the formula

$$Fa = \frac{\sum n^{o}}{N^{o}}$$

Where n^{o}= number of oocytes per female, and N^{o}= total number of females. Oocyte diameter was measured using millimetric graph paper by counting the number of oocytes fitting into 10 mm of the line on the paper and dividing by 10, and later calculating the average number of oocytes on a one-dimensional space for each ovary with oocytes.

RESULTS

Habitat. La Venada Creek is a primary stream in both its highest, and lower sections, with a width of 2-3 m, and a depth 0.5-1 m during both the rainy and dry seasons. Substrate is mostly rocky, with some sand and decomposing vegetation. For most of the length studied shore vegetation is not natural, consisting of white ginger (*Hedychium coronarium*), bamboo (*Guadua angustifolia*), coffee trees (*Coffea arabica*) and banana plantations (*Musa* spp.). Ambient and surface water temperature ranged on average from 18˚C to 20.0°C during low-water season, and from 16.2°C to 20.5°C during the rainy season. Oxygen saturation was around 84% and dissolved oxygen was 6.0mg/L during the dry season, but values were in average higher during the rainy season: 92% and 7.9mg/L respectively. In the dry season pH was near 7.6 and during the wet season 7.1 (Table 1).

Hemibrycon brevispini was found in the middle and lower reaches of La Venada Creek along with *Carlastyanax aurocaudatus* (Eigenmann 1913), *Astroblepus* cf. *cyclopus* Humboldt 1805, *Brycon henni* Eigenmann 1913, *Bryconamericus caucanus* Eigenmann 1913, *Cetopsorhamdia boquillae* Eigenmann 1922, *Chaetostoma* cf. *fischeri* Steindachner 1879, *Parodon caliensis* Boulenger 1895, *Poecilia caucana* Steindachner 1880, *Trichomycterus caliense* Eigenmann 1918 and *T. chapmani* Eigenmann 1918.

Digestive tract morphology. The stomach of *H. brevispini* is longer (mean=12.7 mm, S.D. = 2.79) than wide (mean 7 mm, S.D. = 1.95) and is located in the anterior portion of the coelomic cavity, sometimes thickly covered with fat. Two pyloric caecae are present on the anterior part of the stomach. A significant,

Table 1. Physico-chemical variables during wet and dry season for La Venada Creek, Quindío River drainage, upper Cauca River, Colombia. July 2011 - November 2012. AT: Ambient temperature, WT: Water temperature, DO: Dissolved oxygen, PS: Percent saturation, CO: Conductivity.

	Dry season			Wet season		
	Range	Mean	V (%)	Range	Mean	V (%)
AT (°C)	16.00-20.00	18.00	15.71	14.00-18.46	16.23	19.43
WT (°C)	18.50-21.56	20.03	10.80	18.00-23.00	20.50	17.24
DO (mg/L)	5.00-7.03	6.01	23.86	7.62-8.20	7.91	5.18
PS (%)	83.00-85.00	84.00	1.68	90.00-94.00	92.00	3.07
CO (us)	51.00-62.00	56.50	13.76	67.00-79.00	73.00	11.62
pH	7.01-8.2	7.60	11.06	5.85-8.40	7.125	25.30

positive correlation was found between the intestine length and total length (r=0.67; p=0.017) as well as the intestine length and standard length (r=0.67; p=0,017; Fig. 6).

Diet. The feeding activity of this species is constant throughout the year (emptiness coefficient [V] = 0.82%). Prey abundance did not show normal distribution (p>0.05). The Kruskal-Wallis analysis revealed no difference in the abundance of the diet items between males vs. females or immature vs. adults (KW P= 0.25; df= 1; KW P= 0.31, df= 1 respectively). This was also the case when diet abundances were compared between wet and dry seasons (KW P= 0.98; df= 1). Thus season, sex and maturity are not determinant factors of the prey abundance in the diet of *H. brevispini*, and hence it appears that the items found in its diet are in constant supply all year round. Stones, feathers and nematodes found in the digestive tract of some individuals were considered occasional and accidental.

Stomach contents analysis revealed 41 total prey categories consumed by *H. brevispini* (Table 2, Fig. 2), with Diptera being the most frequently consumed (%N= 11.44; %FO= 16.96; %V= 15.01; IRI= 448.39), followed by Hymenoptera: Formicidae (%N= 15.84; %FO= 13.84; %V= 13.59; IRI= 407.21) and Ephemeroptera: Baetidae (%N= 16.09; %FO= 9.86; %V= 13.44; IRI= 291.19). Occasionally ingested organisms (classified as such based on their low consumption frequency) included Coleoptera, Araneae, Dyctioptera, and other allochthonous material. The degree of digestion of items found in stomach contents and the hour of capture allow us to infer that feeding is diurnal in *H. brevispini*, when they take mostly benthic organisms, some arthropods from the water column or that have fallen into the water from shoreline vegetation. If this species were a nocturnal feeder, the samples made during the day would not have found identifiable stomach contents that for the most part showed little effects of digestion (soft tissue still present) and indication that prey ingestion had occurred shortly before capture.

In the normalized principal component analysis (%PCA) (Fig. 2) of prey item abundances components one (17.18%), two (13.62%) and three (9.33%) explain only 40.14% of total variance. This is a consequence of the elevated heterogeneity of volume of prey consumed and large number of different prey items consumed by different individuals of *H. brevispini.* The principal component analysis recovered only a small percentage of this variability, leaving 59.06% of the variance unexplained. Thus, only a preliminary approximation of the trophic characteristics of this species is possible based on PCA, but it is evident that widely different amounts of a wide variety of prey ítems are eaten. The items Coleoptera, bird feathers, gravel, Diptera, Nematoda and Hemiptera accounted for much of the variation in individual diets, whereas Ephemeroptera, Hymenoptera and others items accounted for little variation and were distributed near the sample centroid. Diptera were very abundant in only a few stomachs and, overall, were not as common as the other items located near the centroid. Most of the individual stomachs in Figure 1 grouped near the origin are indicating that individuals employ a foraging strategy that exploits both dominant and rare prey items. The analysis indicates a broad trophic niche and relatively low between-individual variation in diets. Correlation analysis did not reveal an association between the first axis scores (% PCA1) and fish size (r=0.12, p=0.615), indicating no prey preferences in relation to fish length. ANOSIM revealed non-significant differences between the items found among individuals (R= -0.45, P=1).

Table 2. Diet of *Hemibrycon brevispini* in La Venada Creek, upper Cauca, Colombia. %N= numerical percent, %FO= observed frequency percent, %V= percent volume %I.A. = index of alimentary importance, All: allochthonous, Auto: autochthonous. Only the main items are identified in the table.

Item	Stage	Origin	%N	%V	%FO	IRI
Hymenoptera	-	-	-	-	-	-
Formicidae	Adult	All	**15.83**	**13.58**	**13.84**	**407.21**
Vespidae	Adult	All	0.11	0.24	0.51	0.18
Diptera	Larvae	-	**11.43**	**15.00**	**16.95**	**448.39**
Chironomidae	Larvae	Auto	0.05	0.11	0.17	0.029
Simuliidae	Larvae	Auto	0.28	0.44	1.03	0.75
Psychodidae	Larvae	Auto	0.11	0.32	0.69	0.30
Ceratopogonidae	Larvae	Auto	1.29	1.76	1.73	5.29
Calliphoridae	Larvae	Auto	0.02	0.03	0.17	0.01
Culicidae	Larvae	Auto	1.01	1.29	2.42	5.58
Dixidae	Larvae	Auto	0.59	1.02	1.21	1.96
Muscidae	Adult	All	0.08	0.22	0.51	0.16
Hemiptera	-	-	-	-	-	-
Heteroptera	Adult	All	0.50	0.66	1.73	2.03
Auchenorrhyncha	Adult	All	0.08	0.14	0.34	0.08
Coleoptera	Adult	Auto	2.47	3.06	6.05	33.60
Hydrophilidae	Adult	Auto	0.11	0.26	0.69	0.25
Ptilodactylidae	Adult	All	0.22	0.55	1.03	0.81
Lampyridae	Larvae	Auto	0.05	0.06	0.17	0.021
Gyrinidae	Adult	Auto	0.02	0.04	0.17	0.01
Chrysomelidae	Adult	All	0.05	0.39	0.17	0.07
Ephemeroptera: Baetidae	Nymph	Auto	**16.08**	**13.43**	**9.86**	**291.19**
Odonata	-	-	-	-	-	-
Anisoptera	Nymph	Auto	0.42	1.07	1.73	2.593
Zygoptera	Nymph	Auto	0.11	0.53	0.69	0.45
Trichoptera	-	-	-	-	-	-
Hydropsychidae	Larvae	Auto	1.97	6.13	6.74	54.73
Helicopsychidae	Larvae	Auto	0.05	0.13	0.17	0.032
Hydrobiosidae	Larvae	Auto	0.11	0.52	0.69	0.43
Lepidoptera	Adult	All	0.19	0.58	1.03	0.81
Miriapoda: Diplopoda	Adult	All	0.19	0.63	0.86	0.72
Crustacea: Isopoda	Adult	Auto	0.14	0.39	0.86	0.46
Arachnida: Araneae	Adult	All	0.05	0.09	0.34	0.05
Nematoda (Parasite)	-	Auto	0.33	1.45	1.38	2.47
Gastropoda	-	Auto	0.02	0.24	0.17	0.04
Vegetal Material	-	-	-	-	-	-
Seeds	-	All	2.08	1.30	1.03	3.52
Vegetative tissue	-	All	0.16	0.88	0.86	0.90
Pteridophyta	-	All	0.02	0.04	0.17	0.01
Cyanophyceae (*Oscillatoria* sp.)	-	Auto	-	1.66	1.03	1.72
Feather	-	All	0.14	0.29	0.69	0.30
Oocytes	-	Auto	0.25	0.16	0.34	0.14
Scales	-	Auto	0.02	0.06	0.178	0.01
Dyctioptera: Ootheca	-	All	0.02	0.06	0.17	0.01
Rocks	-	Auto	0.87	0.57	2.42	3.50

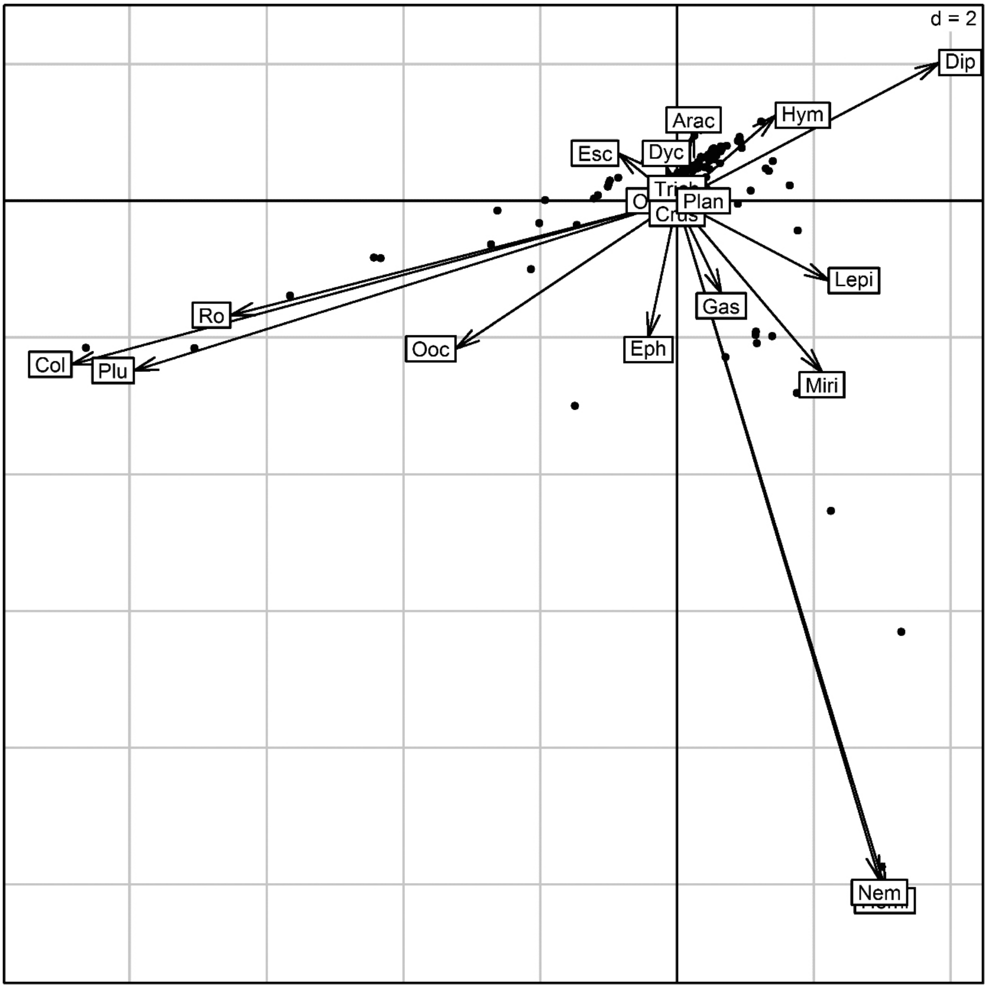

Figure 2. Biplot of prey and stomachs obtained from a Centered Principal Component Analysis (first factorial plane) for *Hemibrycon brevispini*, in La Venada Creek, Quindío River. Dots represent stomachs. Where, Hym: Hymenoptera, Dip: Diptera, Hemi: Hemiptera, Col: Coleoptera, Eph: Ephemeroptera, Odo: Odonata, Trich: Trichoptera, Lepi: Lepidoptera, Miri: Miriapoda, Crus: Crustacea, Arac: Aracnae, Nem: Nematoda, Gas: Gastropoda. Plan: Plantae, Plu: Feather, Ooc: Oocyte, Esc: Escale, Dyc: Dyctioptera, Ro: Rocks.

Condition factor (K). In adults (female), the lowest K values were obtained from July 2011 (dry season), November-December 2011 and October 2012, these coincide with the wet season when fewer prey items were found in stomachs; low K values were obtained for adult males in September 2011 (wet season), February 2012 and July 2012 both of which coincide with the dry season. Maximum K values were obtained for females in October 2011, February and June 2012, during the wet and dry seasons, respectively; maximum K values for males were observed in July 2011 (dry season), October 2011 and 2012, both during the wet season. The lowest and maximum values in condition factor K contrast with the gonadosomatic index value GSI (Fig. 4) and cannot be interpreted as related to gonadal development. In immature specimens, variation was observed in condition factor values with the notable increase occurring between May and November of 2012 and July 2011, which corresponds to the wet and dry season; the variation in K values is considerable in both immature and adults. Comparison of maximum and minimum values by sex for both adults and immatures does not reveal great differences, but lowest values are from April 2012 and highest just one month later in May of 2012.

Reproduction. The gonosomatic index (GSI) (Fig. 4) showed high variation, up to one order of magnitude, among months of this study. *Hemibrycon brevispini* reproduction has three peaks during the year, with two large peaks in GSI in December 2011 and August 2012, and another smaller peak in April 2012.The two larger peaks coincide with the transition from wet to dry season (December) and from dry to wet season (August), and the smaller peak (April) is during the rainy season (Fig. 1). Females had higher GSI values than males throughout the year.

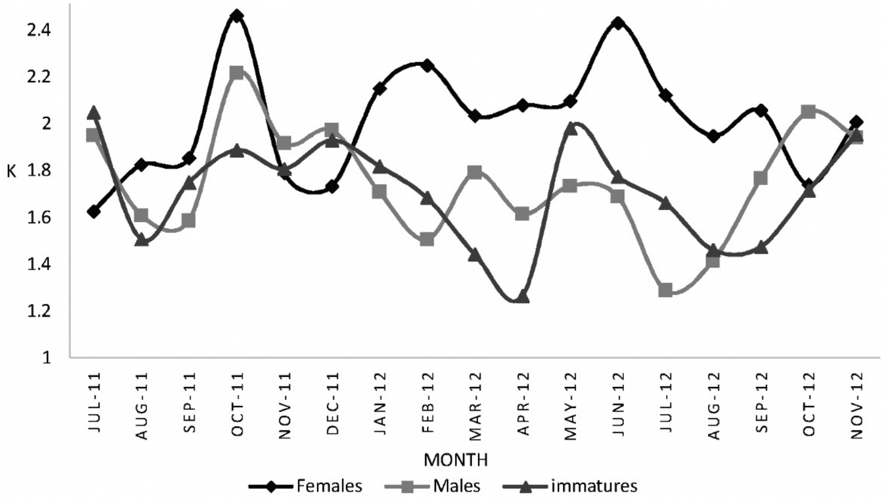

Figure 3. Condition factor (K) of *Hemibrycon brevispini*, in La Venada Creek,Quindío River drainage, upper Cauca, Colombia. July 2011 - November 2012.

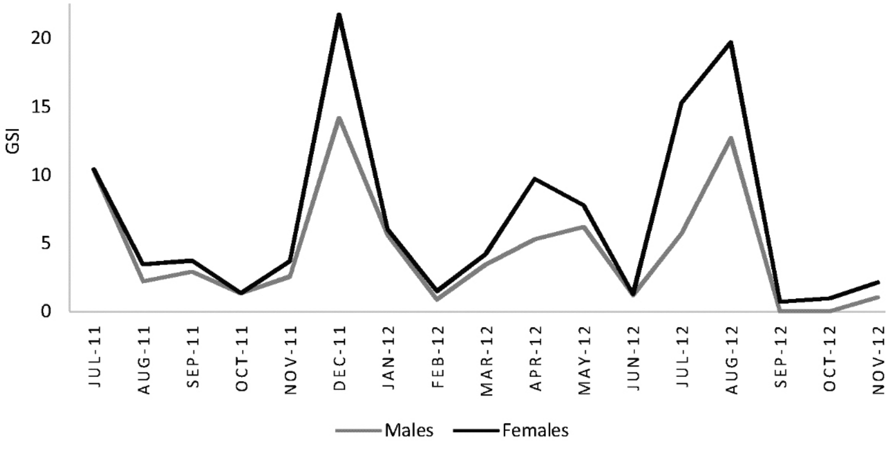

Figure 4. Mean gonosomatic index (GSI) for male and female *Hemibrycon brevispini*, in La Venada Creek,Quindío River drainage, upper Cauca, Colombia. July 2011 - November 2012.

The high number of males present in the population is remarkable: 65.2% were males and 34.7% of the population individuals were females, giving a sex ratio of 1.9 with a predominance of males during the entire study period; significant differences exist as a result ($X^2 = 9.26$, df= 1, p= 0.05). For females, the size at sexual maturity is 76.3 mm SL and for males 68.7 mm SL (Fig. 5). Moreover, the size difference between the sexes is statistically significant (KW, P< 0.05, df= 1).

Fecundity. Average fecundity was 776 oocytes, and the mean diameter of mature oocytes was 0.85 mm (S.D. = 0.26). A non-significant low correlation value was found between fecundity and SL (r= 0.1, P> 0.05, Fig. 6). The mean weight of an oocyte was $3.4 \mathbf{x} \mathbf{10}^{-4}$ g (S.D. =$1,37 \mathbf{x} \mathbf{10}^{-6}$). Total body weight was significantly and positively correlated with gonad weight (r= 0.66, p= 0.019).

DISCUSSION

In streams of the upper Cauca River drainage, two species of *Hemibrycon* feed heavily on benthic insects such as Ephemeroptera, Odonata, and Trichoptera (Román-Valencia & Botero 2006, Román-Valencia *et al*. 2008), and this was also found for *H. brevispini*. Similar diets have been reported for other characid genera of the upper Cauca River drainage such as *Creagrutus brevipinnis* (Román-Valencia 1998), *Roeboides dayi* (Román-Valencia *et al*. 2003), *Argopleura magdalenensis* (Román-Valencia & Perdomo 2004), *Carlastyanax aurocaudatus* (Román-Valencia & Ruiz 2005), and *Bryconamericus caucanus* (Román-Valencia & Muñoz 2001a, Román-Valencia *et al*. 2008). It is commonly accepted that immature aquatic stages of insects are an abundant alimentary resource in Neotropical montane streams; however *H. brevispini* also consumed large amounts of ants (terrestrial Hymenoptera).

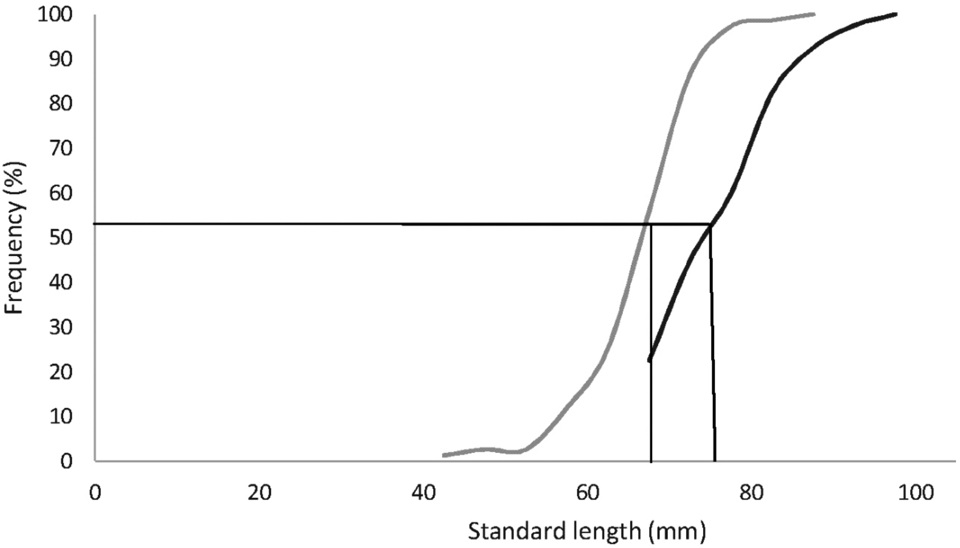

Figure 5. Size at sexual maturity distribution for male and female *Hemibrycon brevispini*, in La Venada Creek,Quindío River drainage, upper Cauca, Colombia. July 2011 - November 2012.

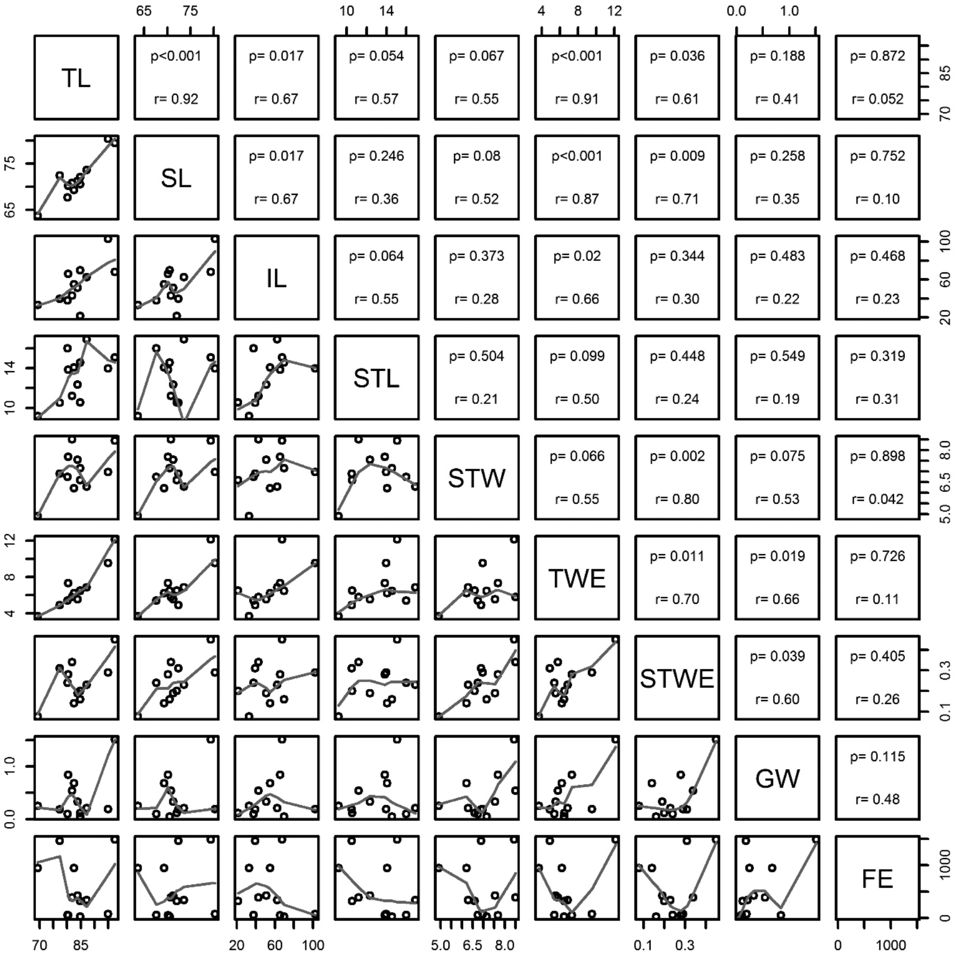

Figure 6. Scatter plot matrix between morphometric variables. The lower panel corresponds to the scatter plot. Upper panel indicates the correlation coefficient and the significance. Abbreviations: standard length (SL), total length (TL), intestine length (IL), stomach width (STW), stomach length (STL) and stomach weight (STWE), gonads weight (GW) and fecundity (FE), stomach width (STW) and total weight (TWE).

Pools and other habitats with low current velocities (0.23 to 0.67 m/s, mean= 0.35 m/s) in La Venada Creek inhabited by *H. brevispini* tended to have more riparian vegetation that probably supports ants and other terrestrial arthropods. Field observations of riparian vegetation in La Venada Creek and other similar Andean streams indicate that areas with lower water velocity are often wider than swift-water reaches and support denser riparian vegetation that in turn offers refuge and food for fishes.

Among allochthonous items found in their diet (14 of 42 categories), ants (Hymenoptera: Formicidae) were much more important than other categories (e.g., Vespidae, Diptera: Muscidae, Heteroptera, Auchenorrhynca, Chrysomelidae, Ptilodactylidae, Lepidoptera, Miriapoda: Diplopoda, Arachnida: Araneae, Seeds, Vegetative tissue, Feather, Pteridophyta, Dyctioptera).

Similar to the observed diet of *H. brevispini*, Román-Valencia *et al*. (2008) reported that *Hemibrycon boquiae* consumed a large proportion of Diptera larvae (Chironomidae, Simuliidae, Tipulidae, Ceratopogonidae and Muscidae): to evaluate why some items but not others are found in stomach contents it would be necessary to conduct prey preference and relative abundance studies, not stomach content occurrence as we present here. However, the ecological characteristics of Neotropical dipterans coincides with the preponderance of exploitation of these prey by *H. brevispini*. Diptera have pupae and larvae with aquatic or semi-aquatic habitats, in both running and quiet waters (Foote 1987, Brown 2001, Merritt *et al.* 2003, Courtney & Merritt 2008, Courtney *et al.* 2008). Among Diptera, Nematoceran families (especially Tipuloidea and Chironomoidea) are a preponderant component of aquatic communities, frequently eaten by primary consumers. Armitage *et al.* (1995) mention that Chironomidae are one of the most abundant families present in freshwater habitats, making them prone to capture.

We also found nematodes in *H. brevispini*, and interpret from their intact state that they are parasites and not prey; which coincides with findings reported for *Bryconamericus caucanus* (Román-Valencia & Muñoz 2001a). The relationship of intestine and body length is a general diet indicator. Herbivores have relatively long intestines compared to carnivores and omnivores (Wootton 1992, Kramer & Bryant 1995). The relatively short intestine length of *H. brevispini* indicates carnivory. *Hemibrycon brevispini* consumed mostly benthic arthropods (35 of the 45 food categories), and plant material was rare in stomachs.

The spawning period for *H. brevispini* differs greatly from patterns reported for other species in the genus. *Hemibrycon boquia*e spawns from July to September, the transition from dry to wet season (Román-Valencia *et al*. 2008). *Hemibrycon quindos* spawns from March to September in both wet and dry seasons (Román-Valencia & Botero 2006) (Fig. 1). Variation observed in the gonosomatic index of *H. brevispini*; could be caused by a seasonal life history (Winemiller 1989, 1992) influenced by a seasonal increase in food availability.

The low abundance of females during the sampling period could be due to bias imposed by males in sex ratios, since according some authors (Daiber 1977, Morse 1981, Petrie 1983, Clutton-Brock 1988, Goto *et al*. 1999, Goto *et al.* 2000, Trivers 1972) this could be a consequence of protandria, in which later maturation of females and dominance by males during juvenile life phases causes differential early mortality. Temperature affects are often cited as a principal cause of sex ration bias, but, the differential reproductive success between sexes or sampling bias have also been indicated as possible explanations. So our results may be a consequence of protandria and later maturation in females. Size at sexual maturity was larger for females than that of males. This may be a strategy of higher initial investment in somatic body weight which allows an increased investment in gonads at a later time. This trade-off strategy implies an extension of the time transcurred before entering the reproductive life phase, which in turn may permit higher mortality before reproduction. Although fitness in one hand is augmented by morphological characteristics, it is at the same time reduced by the inverse relationship between development time and the possibility of death.

Winemiller (1992) noted that medium-sized characids usually have a periodic life strategy characterized by late maturity, large numbers of eggs and low survival. Mean fecundity (776 oocytes) for *H. brevispini* is high when compared to other species of the genus: *H. boquiae* (376 oocytes) (Román-Valencia *et al.* 2008) and *H. quindos* (445 oocytes) (Román-Valencia & Botero 2006). When compared to other characids in the area, *H. brevispini* has lower fecundity than *Bryconamericus caucanus* (3759) (Román-Valencia & Muñoz 2001a), *B. galvisi* (1391 oocytes) (Román-Valencia & Muñoz 2001b), and *Creagrutus brevipinnis* (613 oocytes) (Román-Valencia 1998), but it has higher fecundity than *Carlastyanax aurocaudatus* (181 oocytes) (Román-Valencia & Ruiz-C. 2005).

The periodic life strategy is also evident in the larger average size of females with respect to males, a tendency shared with other species of characids common in the area. A larger size at sexual maturity was found for *H. brevispini* (76.3 mm SL for females and 68.7 mm SL for males) when compared to other species of the genus: *H. boquiae* (65 mm SL for females and 45 mm SL for males) (Román-Valencia *et al.* 2008) and *H. quindos* (53 mm SL for females and 50 mm SL for males) (Román-Valencia 1998); and when compared to other characids in the area: *C. brevispinnis* (40 mm SL) (Román-Valencia 1998), *Bryconamericus caucanus* (50 mm SL for female and 40 mm SL for male) (Román-Valencia *et al.* 2008), *Carlastyanax aurocaudatus* (35 mm SL for female and 40 mm SL for male) (Román-Valencia & Ruiz-C. 2005), except for *B. galvisi* (57.5-89.9 mm SL for female and 61.3-81.1 mm SL for males) (Román-Valencia & Muñoz 2001b).

Although we found no statistically significant seasonal differences in water quality parameters measured (pH, temperature, dissolved oxygen) between the wet and dry seasons (see also other data and parameters measured in Román-Valencia *et al.* 2005), this does not mean that changes in these parameters do not affect *Hemibrycon brevispini*. Physico-chemical parameters for habitats of *H. boquiae* (Román-Valencia *et al.* 2008), *H. quindos* (Román-Valencia & Botero 2006) and *Hyphessobrycon poecilioides* (García-Alzate & Román-Valencia 2008) also showed few significant seasonal differences.
Although physico-chemical parameters measured do not yet indicate decreased water quality in La Venada Creek (see also other data in Román-Valencia *et al.* 2005), threats not evaluated here, occurring near the study site from mining and current cultivated areas make this endemic species' future uncertain. In this study area, other endemic species of *Hemibrycon* are present: *H. boquiae, H. palomae* and *H. quindos,* and are threatened in similar ways as *H. brevispini*. Studies of trophic and reproductive ecology of this species will provide a useful baseline for future impact studies.

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