



Composition and distribution of fishes from the Perequê-Açu river basin, Paraty, Rio de Janeiro, Southeastern Brazil

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Abstract: The large number of less-known coastal drainages in the Atlantic Forest still represents an important gap in the knowledge of ichthyofauna. It is evaluated the taxonomic diversity, frequency and constancy of occurrence, and the distribution of species in the various segments of the Perequê-Açu river basin, state of Rio de Janeiro, Brazil. Twelve sampling campaigns resulted in 13,423 individuals, belonging to seven orders, 10 families, 21 genera, and 23 native species. Cyprinodontiformes and Siluriformes represented about 87.2% of the total abundance, mainly due to the contribution of *Phalloceros anisophallos* and *Schizolecis guntheri*, which account for 63.2% of the total sampled individuals. The analysis of constancy of occurrence reveals that 78.3% of the drainage species are residents, with approximately 93% of the residents of the Carrasquinho River sub-basin. The Sertões River sub-basin had the largest number of species in total: 18. The rarefaction curve did not reach an asymptote, although Chao 2 and bootstrap estimate the richness of 23.9 species and 23.7 species, respectively. The results of PERMANOVA, PCoA, and SIMPER indicate that the fish composition along the catchment is not homogeneous. The conservation status of species is also discussed, regionally, nationally, and globally.

Keywords: Atlantic Rainforest, Conservation, Freshwater fishes, Inventory, Serra da Bocaina National Park.

Composição e distribuição de peixes da bacia do rio Perequê-Açu, Paraty, Rio de Janeiro, Sudeste do Brasil

Resumo: O grande número de drenagens costeiras pouco estudadas na Mata Atlântica ainda representa uma lacuna importante no conhecimento da ictiofauna. Avalia-se aqui a diversidade taxonômica, frequência e constância de ocorrência e a distribuição das espécies nos vários segmentos da bacia do rio Perequê-Açu, estado do Rio de Janeiro, Brasil. Doze campanhas de amostragem resultaram em 13.423 indivíduos, pertencentes a sete ordens, 10 famílias, 21 gêneros e 23 espécies nativas. Cyprinodontiformes e Siluriformes representaram cerca de 87,2% da abundância total, devido sobretudo à contribuição de *Phalloceros anisophallos* e *Schizolecis guntheri*, que respondem por 63,2% do total de indivíduos amostrados. A análise de constância de ocorrência revela que 78,3% das espécies da drenagem são residentes, sendo cerca de 93% dos residentes da sub-bacia do rio Carrasquinho. A sub-bacia do rio Sertões apresentou o maior número de espécies no total: 18. A curva de rarefação não atingiu uma assíntota, embora Chao 2 e bootstrap estimem a riqueza de 23,9 espécies e 23,7 espécies, respectivamente. Os resultados de PERMANOVA, PCoA e SIMPER indicam que a composição dos peixes ao longo da bacia hidrográfica não é homogênea. Discute-se, ainda, o status de conservação das espécies, regional, nacional e globalmente.

Palavras-chave: Conservação, Inventário, Mata Atlântica, Parque Nacional da Serra da Bocaina, Peixes de água doce.

Introduction

The coastal area in the south of Rio de Janeiro state is characterized by presenting relatively narrow stretches of flat terrains located at the base of Serra do Mar (Guerra et al. 2013). The eastern slope of these mountains, the eastern edge of the Brazilian Plateau, is full of relatively short and fast rivers that generally run through a few dozen kilometers till the Atlantic Ocean (Andreata et al. 2002, Buckup 2011, Guerra et al. 2013). Ilha Grande Bay Hydrographic Region consists of independent river basins located in the municipalities of Angra dos Reis and Paraty. These areas are expected to exhibit relatively low diversity but high levels of endemism (Bizerril 1994, Ribeiro 2006, Abilhoa et al. 2011, Buckup 2011). Abell et al. (2008) called this as Ecoregion 325, Fluminense, with endemism rates of 29-39% (at least 28 endemic freshwater fish species). However, most studies approach marine environments (e.g. Andreata et al. 2002, Ferreira et al. 2007, Gaelzer et al. 2007, Amaral et al. 2018), while few studies about freshwater fishes are available (e.g. São-Thiago 1990).

Recent studies (Guerra et al. 2013) show the environmental fragility of this area. The first major impacts in this region were due to urban growth occurred after the construction of Rio-Santos (BR-101), a great scenic beauty road that connects the two biggest South American cities, São Paulo and Rio de Janeiro. Now, the newly paved stretch of Paraty-Cunha road (RJ-165) should increase the population density in this region, increasing negative impacts on watercourses, a fundamental resource both for the good quality of life in the region and for the maintenance of economic activities in that area of the state (Formiga-Johnsson 2015). Decree No. 5,092 / 2004 (Brasil 2004) recommended to a committee working in a Project for Conservation and Sustainable Use of Brazilian Biological Diversity (Probio), the evaluation of priority areas for conservation in the coastal and marine areas of Brazil; one of the extremely high priorities selected areas was exactly Ilha Grande Bay (Creed et al. 2007), although just its marine area. However, the lack of knowledge about the hydrographic basins of this region shows that we are not even able to assess the conservation status of these drainages. The most worrying aspect of this is that in addition to an important ecological role, much of the coastal region in the south of the state is supplied by these rivers, which play a fundamental role in the quality of life and economic activities developed in the region, including ecotourism and adventure tourism (Formiga-Johnsson et al. 2015). Deforestation and pollution caused by inadequate tourism infrastructure intensify the water demands, the uses of rivers and beaches, and is a specific environmental problem of this region (Gaelzer et al. 2007).

Ichthyofauna surveys are extremely important for management and conservation of freshwater fishes (Azevedo-Santos et al. 2019), serving as a first step as they provide the basis for future studies in other areas such as ecology, conservation, and systematics (Frota et al. 2019), as well as being the basis for the description of new species. The Neotropical region has the largest number of freshwater fish species on the planet, with circa of 5,000 species of fishes described, about 3,000 of them in Brazil (Reis et al. 2016). Buckup et al. (2007) pointed out that the number of descriptions of freshwater fish species has been increasing by over 20% annually, especially in the South and Southeast regions of Brazil. According to Langeani et al. (2009), such diversity reflects the complexity of habitat and the life history of freshwater fishes.

The evaluation presented here is a result of a wildlife monitoring project developed during the paving works of the Rio de Janeiro

stretch of Paraty-Cunha road (RJ 165), which crosses Serra da Bocaina National Park. The purpose of the monitoring project was to identify specimens from fauna and flora and potential problems affecting them caused by the engineering work. Thus, the objective of this paper is to inventory the fish species of the Perequê-Açu river basin and its sub-basins, assess their conservation status, and identify the presence of resident, rare and accessory species. As the complexity of the community along the river is expected to increase from upstream to downstream (Vannote et al. 1980), we seek to determine whether the fauna of the high, medium and low stretches of each sub-basin are related to each other.

Material and Methods

1. Study area

The Perequê-Açu river basin (Figure 1) is part of the Ilha Grande Bay Hydrographic Region. Most of the headwaters of the basin are sheltered within Serra da Bocaina National Park (PNSB), the largest Brazilian coastal Park (104,000 hectares; IBAMA 2001), spread between the states of Rio de Janeiro and São Paulo. The Park presents dense ombrophilous forest, mixed ombrophilous forest, and subtropical highland grasslands, with altitudes that range from zero to 2,088 m, which confers to this conservation unit a great diversity and complexity of environments (IBAMA 2001). The climate in the region is temperate super humid, with annual precipitation of 1800 mm. The average temperature is around 16 °C, with high variation between altitude extremes: 38 °C in lowland areas and 0 °C in the highest areas (Guimarães et al. 2000).

The name by which this drainage is known, Perequê-Açu, refers only to the resulting river in the final stretch of this basin. For descriptive and analytic purposes, this drainage was thus separated into five sub-basins that represents its main tributaries, hereafter referred as Sertões river sub-basin (SBS), Pedra Branca river sub-basin (SBP), Muricana river sub-basin (SBM), Carrasquinho river sub-basin (SBC) and Perequê-Açu River itself (SBA).

2. Sampling and data analyses

Twelve samplings of fishes were performed from June 2013 to November 2016 under collecting permit 248/2013 (Ibama, Process 02001.003937/2008-18). Twenty-four georeferenced locations (Table 1) were sampled for 45 minutes each site, in distinct environments with altitudes ranging from 9 m to 1,044 m in stretches of variable length. We used hand nets, sieves, and two trawls: 1.5m long x 1m wide, with 5mm mesh, and 3m long x 2m trawl, with 5mm mesh. Most individuals were identified in the field, counted, and released. Individuals with taxonomic doubts were anesthetized in a 10% eugenol solution, immediately placed in a 10% formalin solution, and then permanently stored in 70% alcohol. Specimens for genetic analysis were immediately deposited in absolute ethanol. The material was deposited in the fish collection at Faculdade de Formação de Professores, Universidade do Estado do Rio de Janeiro (FFP/UERJ) and voucher material was deposited in the ichthyological collection of Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ). Taxonomic identification followed specialized literature and classification adopted is summarized in Fricke et al. (2020).

Fishes from the Perequê-Açu river basin

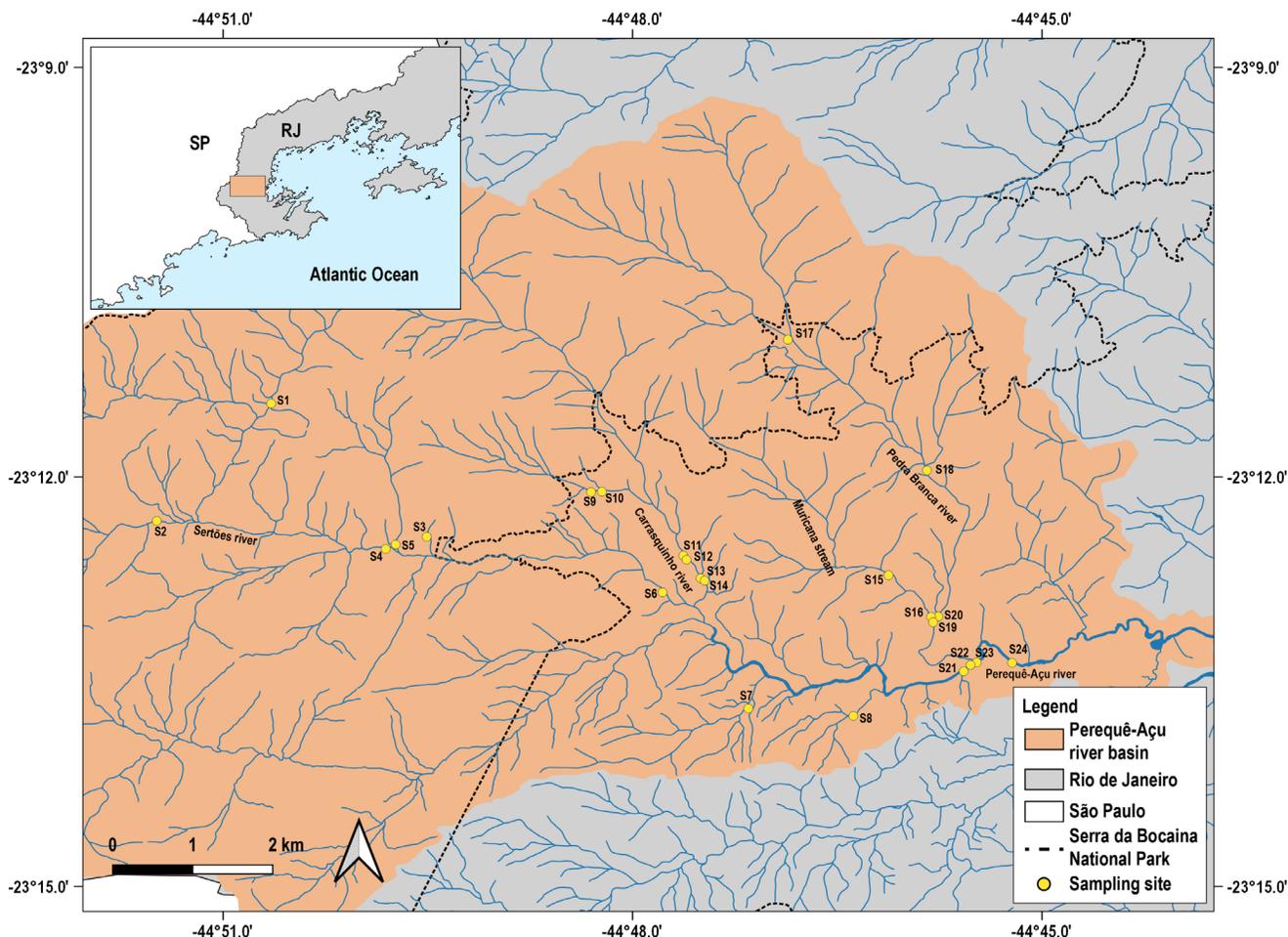


Figure 1. Sampled sites in the Perequê-Açu river basin. SP = São Paulo; RJ = Rio de Janeiro.

A constancy of occurrence index (Dajoz 1983) was applied to analyze the total amount of species obtained in the 12 campaign samples and for the total obtained in each sub-basin to verify the frequency of species in the drainage and in each group of sites. According to this index, constant species are present in more than 50% of the samples, accessory species are present in 25 to 50% and rare species are present in less than 25%. The number of species collected was verified using a rarefaction curve, which is a plot of the number of species as a function of the number of samples, to standardize the species richness expected to be found in a certain number of samples (Melo 2008). We calculated the bootstrap and Chao 2 species estimators, species extrapolation methods to verify whether the maximum number of species has already been sampled in the basin (Efron 1979, Gotelli & Colwell 2011). Additionally, we applied four indices for each site to evaluate the structure of the ichthyofaunistic diversity: Shannon's diversity (H'), Simpson's dominance (D'), Pielou's equitability (J') and total richness (Simpson 1949, Pielou 1975, Krebs 1999, Magurran 2004).

Spatial variations on the ichthyofauna distribution among upper, middle, and lower sites of the basin followed Cavalheiro & Fialho (2020), where further explanations on these procedures can be found. Once the abundances of the species were log-transformed, we performed an a priori Permutational Multivariate

Analysis of Variance (PERMANOVA), based on the Bray-Curtis dissimilarity index, to identify patterns of distribution of species, which if identified were plotted in a Principal Coordinates Analysis (PCoA) ordination graph, using the Bray-Curtis dissimilarity index (Legendre & Legendre 1998). In order to further assess species distribution patterns in the basin, we performed a Similarity Percentage (SIMPER) analysis (Clarke 1993), identifying species that most contributed to dissimilarities between groups of upstream, middlestream and downstream sites.

The rarefaction curve, diversity indices, and species estimators were calculated using the statistical software PAST v. 4.03 (Hammer et al. 2001). The PERMANOVA and SIMPER analyses were performed using the software PRIMER 6.1 with PERMANOVA+ (Clarke & Gorley 2006). The PCoA was performed using R Project for Statistical Computing v. 3.6.1 (R Core Team 2019), with the packages "ape" v. 5.4 (Paradis & Schliep 2019) and "adespatial" v. 0.3-8 (Dray et al. 2020), at a confidence level of 95% ($p < 0.05$). The map of the basin was developed in the software QGIS v. 3.12 (QGIS Development Team 2020).

Results

A total of 13,423 specimens were sampled, belonging to seven orders, 10 families, 21 genera, and 23 species (Table 2; Figures 2-3).

Table 1. Sampled localities in the Perequê-Açu river basin, Paraty, RJ. The sub-basin acronym, the acronym for each site, the name of the site, coordinates, and altitude are displayed. SBS = Sub-basin of Sertões river; SBC = Sub-basin of Carrasquinho river; SBL = Sub-basin of Laje river; SBP = Sub-basin of Pedra Branca river; SBA = Sub-basin of Perequê-Açu river.

Sub-basin	Acronym	Site	Latitude (S)	Longitude (W)	Altitude (m)
SBS	S1	Sertões river headwaters I	23° 12' 21.0"	44° 51' 30.8"	1044
	S2	Sertões river headwater II	23° 11' 29.2"	44° 50' 40.5"	932
	S3	Estiva Preta stream	23° 12' 30.5"	44° 49' 45.8"	638
	S4	Middle Sertões river I	23° 12' 27.1"	44° 49' 32.0"	643
	S5	Middle Sertões river II	23° 12' 32.4"	44° 49' 50.1"	647
	S6	Lower Sertões river	23° 12' 51.6"	44° 47' 48.5"	207
	S7	Lavadeiras stream	23° 13' 43.0"	44° 47' 10.8"	150
	S8	Unnamed Perequê-Açu river tributary	23° 13' 46.4"	44° 46' 24.6"	40
	S9	Sousa stream I	23° 12' 07.4"	44° 48' 19.9"	431
	S10	Sousa stream II	23° 12' 07.7"	44° 48' 15.8"	419
SBC	S11	Carrasquinho river I	23° 12' 35.4"	44° 47' 39.3"	191
	S12	Carrasquinho river II	23° 12' 35.4"	44° 47' 39.3"	191
	S13	Carrasquinho river III	23° 12' 45.5"	44° 47' 31.7"	179
	S14	Carrasquinho river IV	23° 12' 46.0"	44° 47' 30.0"	175
SBM	S15	Upper Muricana stream	23° 12' 44.4"	44° 46' 09.2"	117
	S16	Lower Muricana stream	23° 13' 04.3"	44° 45' 49.5"	40
	S17	Upper Pedra Branca river	23° 11' 00.9"	44° 46' 53.5"	442
SBP	S18	Middle Pedra Branca river	23° 11' 58.2"	44° 45' 52.2"	201
	S19	Lower Pedra Branca river	23° 13' 02.9"	44° 45' 48.7"	42
	S20	Unnamed Pedra Branca river tributary	23° 13' 03.2"	44° 45' 47.9"	46
SBA	S21	Upper Perequê-Açu river I	23° 13' 22.8"	44° 45' 30.3"	16
	S22	Upper Perequê-Açu river II	23° 13' 23.7"	44° 45' 29.9"	15
	S23	Upper Perequê-Açu river III	23° 13' 26.6"	44° 45' 36.1"	15
	S24	Middle Perequê-Açu river	23° 13' 22.8"	44° 45' 14.7"	9

The most representative orders in abundance were Cyprinodontiformes (46.8%) and Siluriformes (40.4%), with 11,700 individuals (about 87.2% of the total sampled, Figure 4), due mainly to the contribution of the two most abundant species in the basin: *Phalloceros anisophallos* (n = 5,853, Figure 3R-3S) and *Schizolecis guntheri* (n = 2,636, Figure 3L). These two species accounts for 63.2% of the sampled individuals. The order Siluriformes was the most taxonomically diverse, with 12 species, followed by Characiformes, with four species (17.4%). Gymnotiformes and Mugiliformes were the least representative orders, presenting only one species each (4.3%). Poeciliidae is the most representative of the families, contributing with 46.8% of individuals (n = 6,281), followed by Loricariidae (n = 4,725, 35.2%) and Characidae (n = 1,474, 11%; Figure 4).

The constancy of occurrence index revealed that most species in the basin are constant (*Deuterodon hastatus*, *D. intermedius*, *Hollandichthys multifasciatus*, *Bryconamericus ornaticeps*, *Acentronichthys leptos*, *Rhamdia quelen*, *Rhamdioglanis transfasciatus*, *Hemipsilichthys nimius*, *Kronichthys heylandi*, *Neoplecostomus paraty*, *Schizolecis guntheri*, *Trichogenes longipinnis*, *Trichomycterus* sp., *Geophagus brasiliensis*, *Awaous*

tajassica, *Eleotris pisonis*, *Phalloceros anisophallos*, and *P. harpagos*, Figure 5), with about 78.3% (n = 18) of frequency in the 12 sampling campaigns, followed by the accessory (13%, n = 3) and rare species (8.7%, n = 2). Carrasquinho river sub-basin proportionally presented the highest percentage of resident species, about 93% of frequency (n = 8), followed by Muricana stream (70%, n = 7) and Sertões river (55.5%, n = 10), the latter being also the sub-basin which presented the largest number of species in total: 18.

The site with the highest richness and abundance (S = 13, n = 1,520) was S23, Upper Perequê-Açu river III (Table 3). Sertões river headwater I (S1) presented the lowest richness (S = 2) and abundance (n = 34). Estiva Preta stream (S3) presented the lowest dominance rates of the basin (D = 0.197), as well as the highest values on diversity and evenness (H' = 1.675, J' = 0.935). A near site, Middle Sertões river I (S4), presented lower evenness (J' = 0.123), diversity (H' = 0.171), and high dominance values (D = 0.932). The rarefaction curve did not reach an asymptote (Figure 6), even in the 95% confidence interval. The Chao 2 and bootstrap richness estimators estimate the richness of 23.9 species (± 2.13) and 23.7 species, respectively. Information on the conservation status of the species can be seen in Table 2.

Table 2. Taxa sampled in the Perequê-Açu river basin, Paraty, RJ. The acronym used in analyses, relative frequency (%) of the species, their conservation status, the category of constancy, and the voucher of the ichthyological collection are presented. DD = Data deficient; LC = Least Concern; NT = Nearly threatened; VU = Vulnerable.

Taxa	Acronym	Fr (%)	Conservation Status			Constancy of Occurrence	Voucher PX-FFP
			Rio de Janeiro Red List (2000)	Brazilian Red List (2014)	IUCN (2019)		
Characiformes							
Characidae							
<i>Deuterodon hastatus</i> (Myers, 1928)	Dha	0.54		LC		Constant	1298
<i>Deuterodon intermedius</i> (Eigenmann, 1908)	Din	0.34		LC		Constant	1338
<i>Hollandichthys multifasciatus</i> (Eigenmann & Norris, 1900)	Hmu	1.43		DD		Constant	1189
<i>Bryconamericus ornaticeps</i> Bizerril & Perez-Neto, 1995	Bor	8.68		LC		Constant	1176
Siluriformes							
Heptapteridae							
<i>Acentronichthys leptos</i> Eigenmann & Eigenmann, 1889	Ale	0.69	VU	LC	LC	Constant	1194
<i>Pimelodella lateristriga</i> (Lichtenstein, 1823)	Pla	0.03		DD		Accessory	1156
<i>Rhamdia quelen</i> Quoy & Gaimard, 1824	Rqu	0.17		LC	LC	Constant	0849
<i>Rhamdioglanis transfasciatus</i> Miranda Ribeiro, 1908	Rtr	1.87		LC		Constant	0413
Loricariidae							
<i>Ancistrus multispinis</i> (Regan, 1912)	Amu	0.11		LC		Accessory	1361
<i>Hemipsilichthys nimius</i> Pereira, Reis, Souza & Lazzarotto, 2003	Hni	2.93		NT		Constant	0406
<i>Kronichthys heylandi</i> (Boulenger, 1900)	Khe	7.32	VU	LC		Constant	0424
<i>Neoplecostomus paraty</i> Cherobim, Lazzarotto & Langeani, 2016	Npa	5.01				Constant	0427
<i>Pareiorhina rudolphi</i> (Miranda Ribeiro, 1911)	Pru	0.19	VU	NT		Accessory	1250
<i>Schizolecis guntheri</i> Miranda Ribeiro, 1918	Sgu	19.64		LC		Constant	0408
Trichomycteridae							
<i>Trichogenes longipinnis</i> Britski & Ortega, 1983	Tlo	0.31		NT		Constant	1305
<i>Trichomycterus</i> sp.	Tsp	2.10				Constant	0414
Gymnotiformes							
Gymnotidae							
<i>Gymnotus pantherinus</i> (Steindachner, 1908)	Gpa	0.01		LC		Rare	1154
Cichliformes							
Cichlidae							
<i>Geophagus brasiliensis</i> Quoy & Gaimard, 1824	Gbr	0.94		LC		Constant	0815
Gobiiformes							
Gobiidae							
<i>Awaous tajasica</i> Lichtenstein, 1822	Ata	0.62		LC	LC	Constant	0832
Eleotridae							
<i>Eleotris pisonis</i> (Gmelin, 1789)	Epi	0.14		LC	LC	Constant	0475
Mugiliformes							
Mugilidae							
<i>Mugil liza</i> Valenciennes, 1836	Mli	0.15		NT	DD	Rare	0834
Cyprinodontiformes							
Poeciliidae							
<i>Phalloceros anisophallos</i> Lucinda, 2008	Pan	43.60		LC		Constant	0584
<i>Phalloceros harpagos</i> Lucinda, 2008	Pha	3.19		LC		Constant	1249
	Total	100					



Figure 2. Species sampled in the Perequê-Açu river basin. (A) *Deuterodon hastatus*; (B) *Deuterodon intermedius*; (C) *Bryconamericus ornaticeps*; (D) *Hollandichthys multifasciatus*; (E) *Rhamdia quelen*; (F) *Rhamdioglanis transfasciatus*; (G) *Ancistrus transpinis*; (H) *Hemipsilichthys nimius*; (I) *Kronichthys heylandi*; (J) *Neoplecostomus paraty*.



Figure 3. Species sampled in the Perequê-Açu river basin. (K) *Pareiorhina rudolphi*; (L) *Schizolecis guntheri*; (M) *Trichogenes longipinnis*; (N) *Trichomyterus sp.*; (O) *Geophagus brasiliensis*; (P) *Awaous tajasica*; (Q) *Eleotris pisonis*; (R) female *Phalloceros harpagos*; (S) male *Phalloceros anisophallos*; (T) female *Phalloceros anisophallos*.

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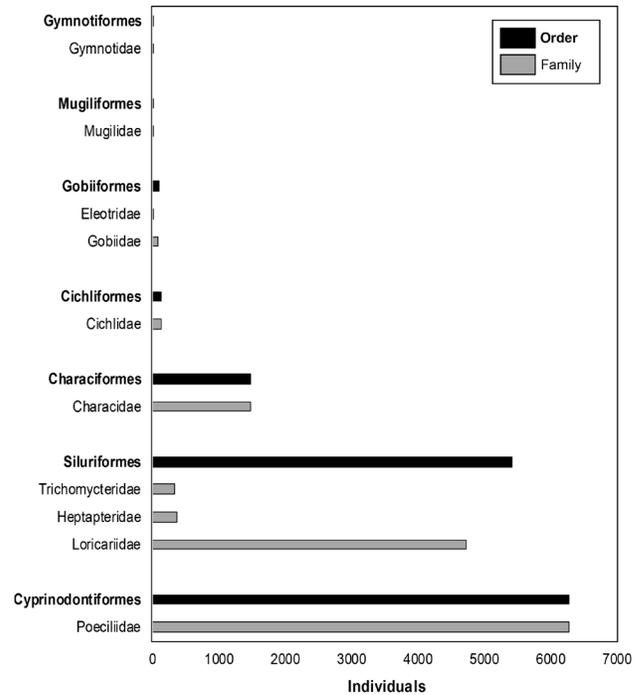


Figure 4. Representativeness graph of the sampled specimens regarding orders and families in the Perequê-Açu river basin.

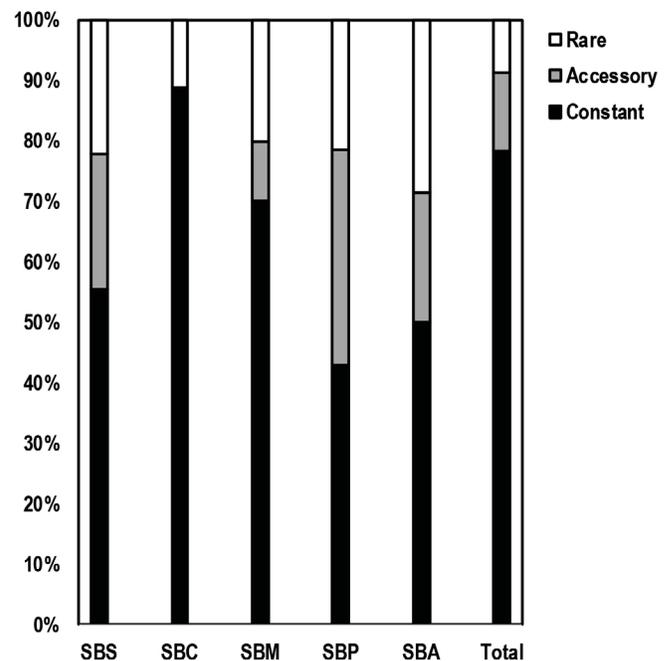


Figure 5. Constancy of occurrence of the Perequê-Açu river basin fish species. SBS = Sertões river sub-basin; SBC = Carrasquinho river sub-basin; SBM = Muricana stream sub-basin; SBP = Pedra Branca river sub-basin; SBA = Perequê-Açu river sub-basin.

The PERMANOVA result indicated that the spatial distribution of the species is not homogenous along the longitudinal gradient of the drainage (Df = 2, Pseudo-F = 4.20, p = 0.0002), revealing differences in the composition of species among upstream, midstream, and

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Table 3. Diversity indexes of the sampling sites. n = total individuals. S = total taxa. D = dominance index. H' = Shannon index. J' = Pielou index.

Site	n	S	D	H'	J'
S1	34	2	0.701	0.451	0.651
S2	81	5	0.494	0.831	0.517
S3	169	6	0.197	1.675	0.935
S4	87	4	0.932	0.171	0.123
S5	486	7	0.451	1.009	0.518
S6	643	10	0.267	1.569	0.681
S7	150	5	0.339	1.165	0.724
S8	217	9	0.458	1.109	0.505
S9	219	3	0.541	0.784	0.714
S10	707	7	0.449	1.170	0.601
S11	442	5	0.478	0.987	0.613
S12	448	8	0.348	1.282	0.616
S13	625	7	0.385	1.135	0.583
S14	419	8	0.329	1.342	0.645
S15	2337	6	0.516	0.913	0.510
S16	849	10	0.363	1.305	0.567
S17	161	5	0.348	1.244	0.773
S18	198	7	0.246	1.484	0.762
S19	460	10	0.291	1.494	0.649
S20	98	6	0.808	0.443	0.247
S21	1179	12	0.407	1.167	0.470
S22	384	10	0.298	1.383	0.601
S23	1520	13	0.507	0.953	0.371
S24	1510	12	0.324	1.383	0.557

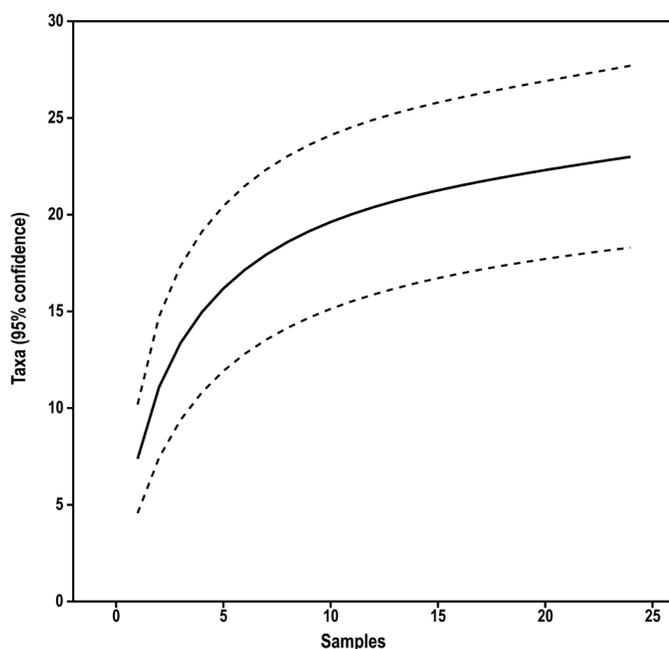


Figure 6. Sample rarefaction curve of the species in the Perequê-açu river basin. The middle line represents the number of sampled species. Dotted lines represent the 95% bootstrap interval.

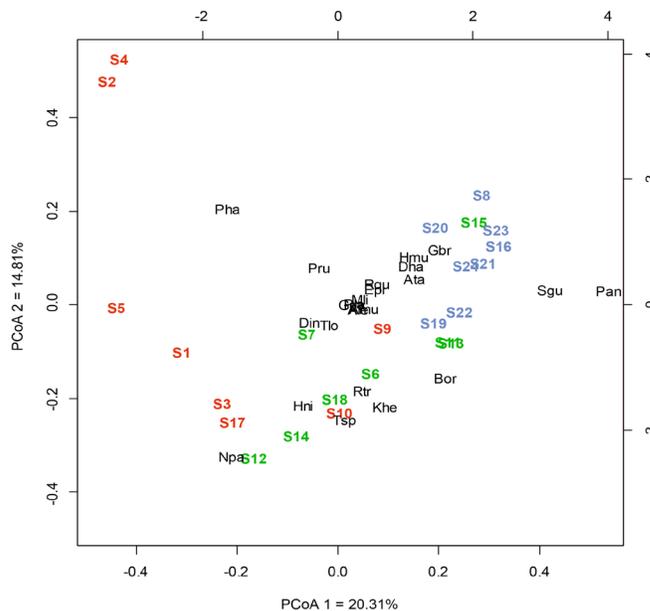


Figure 7. Principal Coordinates Analysis ordination plot, evidencing the composition and distribution of species along upper (red), middle (green) and lower (blue) streams of the Perequê-Açu river basin. List of species abbreviations is presented in Table 2.

downstream sites. Species richness and abundance tended to increase towards downstream: 12 species in upper streams (n = 1,944), 15 in middle (n = 5,262) and 18 in lower streams (n = 6,217). The PCoA segregated groups of streams according to their faunistic composition (Figure 7). *Neoplecostomus paraty*, *Phalloceros harpagos*, and *Pareorhina rudolphi* were associated with the upper portions of the basin. Species associated with intermediate portions of the basin, such as *Kronichthys heylandi*, *Rhamdioglanis transfasciatus*, and *Bryconamericus ornaticeps* inhabit both upper and lower portions of this drainage. *Schizolecis guntheri* and *Phalloceros anisophallos*, very strongly associated with lower portions of the basin. The analysis also grouped species of low representativity and occurrence in the central part of the graph, such as *Gymnotus pantherinus*, *Pimelodella lateristriga*, *Rhamdia quelen*, and *Ancistrus multispinis*. The first axis of the graph (PCoA 1 = 20.31%) showed a pattern of decreasing representativeness of siluriform species in the downstream direction, and an increase in the occurrence of Characiformes and Cyprinodontiformes.

SIMPER shows that for the upper portion of the basin (average dissimilarity = 26.1%), *K. heylandi*, *N. paraty*, *Trichomycterus* sp., and *P. harpagos* are the species that most contribute to differentiate these locations from others; in middle streams (average dissimilarity = 48.4%) *P. anisophallos*, *S. guntheri*, *B. ornaticeps*, *Trichomycterus* sp., and *R. transfasciatus*; and in lower sites (average similarity: 56.3%) *P. anisophallos*, *S. guntheri*, *K. heylandi*, *Geophagus brasiliensis*, and *Awaous tajasica* (Table 4). The highest species composition dissimilarity among groups of locations was between upstream and downstream sites, with an average difference of 80.2%.

Table 4. Contribution (%) of the most relevant species to dissimilarity in each portion of the basin according to the SIMPER analysis.

Species	Groups		
	Upstream	Middlestream	Downstream
Average similarity (%)	26.1	48.4	56.3
<i>Kronichthys heylandi</i>	26.5		12.8
<i>Neoplecostomus paraty</i>	23.8		
<i>Trichomycterus sp.</i>	20.7	13.7	
<i>Phalloceros harpagos</i>	17.8		
<i>Phalloceros anisophallos</i>	3.8	23.3	34.2
<i>Shizolecis guntheri</i>		18.0	26.6
<i>Bryconamericus ornateiceps</i>		14.4	
<i>Rhamdioglanis transfasciatus</i>		12.1	
<i>Geophagus brasiliensis</i>			6.2
<i>Awaous tajasica</i>			6.2

	Groups		
	Up x Middle	Up x Down	Middle x Down
Average dissimilarity (%)	70.5	80.2	57.2

Discussion

It is already well known (e.g. Lowe-McConnell 1999) that the two largest native orders of Otophysi (Characiformes and Siluriformes) from South America are predominant in all Brazilian rivers and streams and, in this case, it was not different. This time, however, since the basin is constituted mostly by mountain streams, which form very steep rapids, the Siluriformes were represented by three times more species than Characiformes, since they fit well in this type of habitat. Loricariidae holds the highest taxonomic diversity of species in the drainage, as their members prefer rapids in bedsides or marginal vegetation in less sloping terrain, recurrent habitats in coastal drainages of the Atlantic Rainforest (Casatti 2005). Among the 12 species of this family, *Kronichthys heylandi* had the widest distribution in the basin, present in 20 of the 24 sampled locations, from the lowest to the highest site. This was also one of the most abundant species identified by Ferreira and collaborators (2019) in the Itanhaém river, although it was classified as a strongly rhithronic species, that is, more common in the high course of streams. Buck & Sazima (1995) report that the species is generalist, presenting a wide variety of eating habits and environments where they can live, clinging to rocks, submerged woods, plants and foraging algae, insect larvae, crustaceans and macrophytes throughout the day, which may explain the presence of this taxon at almost all sites of the drainage.

The most abundant species of the family Loricariidae and the order Siluriformes in the samplings was *Schizolecis guntheri*. This species is widely distributed in coastal basins from the north of Santa Catarina to Rio de Janeiro, adapting to several types of environment with shallow waters and abundant marginal vegetation, regardless of the speed of the water current (Buck & Sazima 1995, Bizerril & Primo 2001, Kusma 2013). The latter author reported an interesting type of sexual dimorphism that only occurs in the populations of *Schizolecis guntheri* in the hydrographic region of Ilha Grande Bay: in other locations females and males of *S. guntheri* have the same color and body size, but in Ilha Grande Bay the males have a bulkier body with many dark

spots, as it could be attested in the specimens collected in the present study. Both *Kronichthys heylandi* and *Schizolecis guntheri* were also very abundant species in the area studied by Esteves & Lobón-Cerviá (2001), but the latter showed greater abundance in the rainy season; in Perequê, it was abundant throughout the year, mainly downstream, also differing from the findings of Ferreira et al. (2019). Also in this family, *Hemipsilichthys nimius* has a narrow distribution, as the other two species of this genus (Buckup 2011): it is endemic to this region, occurring only in the Perequê-Açu and the Taquari river basins, also in Paraty (Pereira et al. 2003, Pereira 2005), where they are relatively common in rapids in middle streams.

The constancy of occurrence analysis revealed that most species in the basin are resident and non-migratory, including when the analysis was applied for each sub-basin. Regarding the constancy of occurrence in the five sub-basins, the one with the highest percentage of rare species was Perequê-Açu, in its final stretch. This result has two reasons: first, it is due to the low representativeness of species that are normally found in the upper and middle reaches of the basin, such as *Bryconamericus ornateiceps* (Figure 2C), *Rhamdioglanis transfasciatus* (Figure 2F) and *Rhamdia quelen* (Figure 2E). Second, as it is the lowest part of the basin, closer to the ocean, it houses peripheral freshwater species like *Awaous tajasica* and *Eleotris pisonis* (Medeiros et al. 2019), rarer in our samples. *Mugil liza*, a marine species, also contributed to this highest rarity value, being found on only one occasion. Vieira (2000) remembers that this index does not consider the abundance of species, only the presence; so, the sampling effort should be distributed throughout all day long (Azevedo-Santos et al. 2019), and also seasonally, to ensure the capture of all species from a site. Alone or associated with the frequency of occurrence, this index can offer an indication of the distribution of species in a given location.

The rarefaction curve indicates that the maximum number of species in the basin may not have been reached, as the curve has not reached an asymptote. This result is in line with the values of species estimators, which also revealed that there is a possible variation in the richness of taxa in the basin. However, the results of both analyses estimate that we were close to the “real” number of species. Possibly, if we had collected in more stretches of the low course of the river, we would have captured species that are commonly obtained in these lowland areas. São-Thiago (1990), in an unpublished dissertation, mentions that she collected almost the same number of species that we collected in Perequê: 22, 13 of which apparently were also collected in the Perequê basin; to be sure, it would be necessary to re-examine the material collected by the author, to update some identifications.

Two species of *Phalloceros* occur in allopatry in this basin, with a clear preference of *P. harpagos* (Figure 3R) for headwaters and bedside environments, and *P. anisophallos* (Figure 3S, 3T), the most abundant species in the basin, which is a very tolerant species (Mazzoni et al. 2011), found in lowland environments with or without marginal vegetation. *P. harpagos* was one of the most abundant species in headwater streams from Paranapanema basin (Frota et al. 2020) or in São José dos Dourados and Turvo-Grande basins (Casatti et al. 2012) where, despite the large area collected, only one species of this genus was recorded.

The low representativeness of Gymnotiformes, otherwise, may happen mainly due to the habits of the species. Like other parts of the Brazilian Crystalline Complex, Serra do Mar has about 50% granitoids

and 30% gneisses (Guerra et al. 2013); thus, the riverbed is sandy and shallow, with the bottom formed by large slabs of stones. This type of habitat does not favor the formation of marginal sites and, allied to the slope of the terrain, the water speed does not seem to be adequate to provide a good habitat for species like *Gymnotus pantherinus*. Nocturnal fishes, during the day they are used to shelter in cracks or holes in clayey or sandy ravines, under stones and submerged vegetation (Crampton & Albert 2006), places of low occurrence in this basin. The only specimen was found in a small, first-order stream on a flat stretch rich in silt; this stream was receiving remnants of construction works, which contributed to its silting up and possibly to the local extinction of the species.

Following the pattern presented in other drainages (e.g. Corrêa et al. 2015, Silva et al. 2016), the species richness increased in the upstream-downstream direction, which is related to the increased diversity and complexity of microhabitats. The rise in richness values due to the increase in the types of environments is in line with what was expected by the River Continuum Concept (Vannote et al. 1980). According to Garutti (1988), as they have a smaller volume of water, the headwaters of the streams do not support a large number of individuals and do not favor the emergence of varied microhabitats, which influences species richness. Modifications in the fish assemblages following the longitudinal gradient of a river are expected, as a result of evolutionary adaptations of each species (Silva et al. 2016).

In this way, PERMANOVA, PCoA, and SIMPER results indicate that the composition of the fishes along the catchment is not homogenous. In the upper reaches of the basin, the substrate of rocks, fast-flowing water in rapids areas, and moderate riparian vegetation seem to be favorable to the greater abundances of Loricariidae species such as *Neoplecostomus paraty*, strongly associated with these environments (Cherobim et al. 2016). On the other hand, these slope headwater habitats limit the great majority of species, mainly Characiformes and Cyprinodontiformes. Middle and lower portions, exhibiting a greater availability of microhabitats, feeding resources, presence of sand and gravel, larger width and depth of watercourses, as well as the increase of marginal vegetation are capable of sustaining more species, especially those with greater abundance and versatility in the basin, such as *P. anisophallos*, *B. ornateiceps*, *S. guntheri*, and *K. heylandi*. The SIMPER result indicates that *P. anisophallos* and *S. guntheri* present an increasing contribution along the longitudinal gradient, being most prominent in the middle and lower reaches of the catchment, places with greater availability of detritus, allochthonous and autochthonous feeding resources (periphyton or animals; Buck & Sazima 1995, Mazzoni et al. 2010, Ceni & Barreto 2018).

The strong dissimilarity between upstream and downstream sites in the catchment is a result of the differences between their fauna and the contribution of each species within a group of streams. However, it is worth mentioning that while species such as *Hemipsilichthys nimius* and *Neoplecostomus paraty* are abundant in median portions of the basin, their frequencies of occurrence in these locations are lower than those of species such as *Bryconamericus ornateiceps* and *Rhamdioglanis trans fasciatus*. Clarke (1993) then suggests that species that do not have a consistent abundance within a group are not good indicators for clusters of sites. Thus, drawing a parallel to the results of the analysis of constancy of occurrence, SIMPER deemed relevant for the dissimilarities only constant species, excluding accessory and rare species (even if they present higher abundance). For downstream

sites, while *Awaous tajasica* showed lower abundance than other species in the same group of sites, it stands out because it occurs with more consistency than others.

All three works dealing with the conservation status of this fauna used the IUCN criteria (Mazzoni et al. 2000, ICMBio 2018 and IUCN 2019). Also, the data that is made available in the publication of ICMBio (2018) are the result of a large number of experts who analyzed each one of the Brazilian continental fish species, producing a detailed and comprehensive list. Most of the 23 species were considered as Least Concern (15); four were classified as are considered as nearly threatened (NT) and on two of them the researchers did not find enough data to apply the criteria (so, they were classified as Data Deficient); from the remaining two, one is recently described (*Neoplecostomus paraty*) and the last is not identified yet (*Trichomycterus* sp.). In the Rio de Janeiro survey (Mazzoni et al. 2000), *Acentronichthys leptos*, *Kronichthys heylandi* and *Pareiorhina rudolphi* are listed as Vulnerable (VU); in ICMBio (2018), these first two species are listed as Least Concern (LC). Globally (IUCN 2019), four species are categorized as Least Concern (LC, *Acentronichthys leptos*, *Awaous tajasica*, *Eleotris pisonis*, and *Rhamdia quelen*) and one as Data Deficient (DD, *Mugil liza*). The advice of ICMBio (2018) is that there should be an effort to expand research to include species about which we do not have sufficient knowledge. Although this seems like something that should be done immediately, in marine and freshwater fish there are 564 species listed as DD. It will take some time for each of these species to be included in a program that involves identifying populations for specimen collections and analysis of taxonomic, trophic and reproductive data, in addition to verifying how structural characters and physiological processes vary over time and in different spaces. There is no guarantee that no species will be lost in the process.

Only seven species are protected under the legislation of the National System of Conservation Units (SNUC), being found within the PNSB: *Deuterodon intermedius*, *Kronichthys heylandi*, *Neoplecostomus paraty*, *Pareiorhina rudolphi*, *Phalloceros harpagos*, *Trichogenes longipinnis*, and *Trichomycterus* sp. Of these species, *K. heylandi*, *N. paraty*, and *Trichomycterus* sp. are also in the buffer zone of the park; the remaining 19 species are not included in any protected area. The river's banks in areas outside the park have already been or are being deforested now, giving way to urban advancement. This implies a loss of habitats outside PNSB and its buffer zone that is expected to simplify the structure of communities (Casatti et al. 2012, Miranda 2012, Teresa & Casatti 2012). This denotes the importance of studies that not only list species or seek to understand how the fish assemblages are organized, but address their conservation status for the improvement of future species lists and implementing practical management actions.

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Author Contributions

Felipe Vieira Guimarães: designed and wrote the manuscript, went to fieldwork, and discussed the results.

Rosana Souza-Lima: designed and wrote the manuscript, went to fieldwork and discussed the results.

Tiago Medeiros de Souza: went to fieldwork and discussed the results.

Richard Rangel Rodrigues: went to fieldwork and discussed the results.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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