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Sampling effort and fish species richness in small *terra firme* forest streams of central Amazonia, Brazil

Maeda Batista dos Anjos* and Jansen Zuanon**

Small streams are important components of the landscape in *terra firme* forests in central Amazonia and harbor a large number of fish species. Nevertheless, the lack of a common sampling protocol in studies of this ichthyofauna hinders comparisons among available results. This study evaluates how the length of stream reach sampled affects estimates of local fish species density in 1st, 2nd, and 3rd order streams, and proposes a mean minimum sampling length that best approximates the absolute number of species in a given stream segment. We sampled three streams in the Biological Dynamics of Forest Fragments Project's study sites, between May and August 2004. At each stream, one 1st order, one 2nd order, and one 3rd order segment was sampled. We sampled five 20-m reaches in each stream segment. Three to four people collected along each reach for 45 to 60 minutes. We used Jaccard's coefficient to estimate the similarity of species composition among stream reaches and segments. Estimates of species richness were obtained with Jackknife 1 and Bootstrap algorithms and species accumulation curves. We used simple linear regressions to look for relationships between species density and fish abundance and between species density and the volume of 100-m stream segments. Species density in 1st order stream reaches was slightly higher than in 2nd and 3rd order stream reaches, whereas fish abundance was apparently higher in 3rd order reaches. Similarity in fish species composition between 20-m reaches was low for all studied streams. Species density values in pooled 100-m stream segments represented 71.4% to 94.1% of the estimated values for these streams. Species density showed a direct relationship both with volume of the sampled stream segment and fish abundance. It seems plausible that larger streams contain a higher number of microhabitat types, which allow for the presence of more fish species per stream length. Based on the values of asymptotes and equations for species accumulation curves, the mean minimum sampling length that best estimates the absolute number of species in a stream segment is 180 m ± 20 sd for 1st order segments; 213 m ± 23 sd for 2nd order segments, and 253 m ± 30 sd for 3rd order stream segments.

Pequenos igarapés são componentes importantes da paisagem das florestas de terra firme da Amazônia Central e abrigam um grande número de espécies de peixes. Contudo, a falta de um protocolo de amostragem comum em estudos desta ictiofauna dificulta a comparação dos resultados obtidos. Este estudo avalia como o comprimento dos trechos amostrados afeta as estimativas de densidade local de espécies de peixes em riachos de 1ª, 2ª e 3ª ordens e propõe um comprimento mínimo médio de amostragem que melhor estima o número absoluto de espécies em um dado segmento de riacho. Nós amostramos três sistemas de riachos nas áreas de estudo do Projeto Dinâmica Biológica de Fragmentos Florestais, entre maio e agosto de 2004. Em cada riacho, foi amostrado um segmento de 1ª ordem, um de 2ª ordem e um de 3ª ordem. Amostramos cinco trechos de 20-m em cada segmento de riacho. Três a quatro pessoas coletaram ao longo de cada trecho durante 45 a 60 min. Usamos o coeficiente de Jaccard para estimar a similaridade da composição de espécies entre trechos e entre segmentos de riachos. Estimativas de riqueza de espécies foram obtidas com uso dos algoritmos de Jackknife 1 e Bootstrap e de curvas de acumulação de espécies. Usamos regressões lineares simples para verificar relações entre a densidade de espécies e a abundância de peixes e entre densidade de espécies e o volume de segmentos de 100-m de riacho. A densidade de espécies foi um pouco maior nos trechos de riacho de 1ª ordem do que nos de 2ª e 3ª ordens, contudo a abundância de peixes foi aparentemente maior nos trechos de 3ª ordem. A similaridade na composição de espécies de peixes entre trechos de 20-m foi baixa para todos os riachos estudados. Valores de densidade de espécies para segmentos de 100-m representaram de 71,4% a 94,1% dos valores estimados para esses riachos. A densidade de espécies mostrou uma relação direta tanto com o volume do segmento de riacho amostrado, como com a abundância de peixes. Parece plausível que riachos maiores contenham um número maior de tipos de microhábitats, que permitiriam a presença de mais espécies de peixes por trecho de riacho. Baseado nos valores das assíntotas e nas equações das curvas de acumulação de espécies, o comprimento mínimo médio de amostragem que melhor estima o número absoluto de espécies em um segmento de riacho é 180m ± 20 dp para segmentos de 1ª ordem; 213 m ± 23 dp para os segmentos de 2ª ordem e 253 m ± 30 dp para os segmentos de 3ª ordem.

Key words: fish diversity, fish abundance, stream size, species composition, species accumulation curves.

*Programa de Pós-Graduação em Biologia Tropical e Recursos Naturais, Instituto Nacional de Pesquisas da Amazônia /Universidade Federal do Amazonas, Manaus, AM, Brazil. maedaangel@hotmail.com

**Coordenação de Pesquisas em Biologia Aquática, Instituto Nacional de Pesquisas da Amazônia, Manaus, AM, Brazil. zuanon@inpa.gov.br

Introduction

Small water courses are important components of the landscape in *terra firme* (*i.e.*, non-flooding) forests in central Amazonia. Canopies of adjacent trees cover a large proportion of these streams' beds, known regionally as *igarapés*. The aquatic fauna is largely dependent on organic matter from the surrounding terrestrial system, in the form of flowers, fruits, leaves, and invertebrates (Goulding, 1980; Walker, 1991; Lowe-McConnell, 1999). These small streams shelter a large number of fish species, mainly small Characiformes (Sabino & Zuanon, 1998; Lowe-McConnell, 1999; Buhrnheim, 2002; Buhrnheim & Cox-Fernandes, 2003).

Although widespread in the Amazon basin, this ichthyofauna is still poorly known. The majority of published works have focused on activity periods, feeding habits, and community structure of these fishes (Silva, 1993, 1995; Sabino & Zuanon, 1998; Araújo-Lima *et al*., 1999; Buhrnheim & Cox-Fernandes, 2001; Buhrnheim, 2002; Buhrnheim & Cox-Fernandes, 2003; Zuanon & Sazima, 2004; Mendonça *et al*., 2005). These studies applied diverse sampling techniques and fishing gears, and differed in stream reach sampling length, sampling effort, sampling period (day/night), and use of auxiliary sampling techniques, such as underwater observation by snorkeling. The lack of a common sampling protocol in studies of this ichthyofauna hinders comparisons among available results. Uncertainty about the effectiveness of sampling techniques in these streams raises doubts about the patterns of species occurrence and distribution revealed in such studies. Small sample sizes may not include rare species that are important in the composition of fish communities, resulting in misleading estimates of species richness (Angemeier & Smogor, 1995; Cao *et al*., 1998). Also, poorly outlined sampling protocols may generate an underestimate of alpha diversity, while at the same time overestimating beta diversity (Santos, 2003), which could impair efforts to establish conservation strategies for this ichthyofauna (Mendonça *et al*., 2005).

This study evaluates the effect of the length of stream reach sampled on estimates of local fish species richness (*i.e.* species density; Gotelli & Cowell, 2001), and proposes a mean minimum sampling length that best estimates the absolute number of species in a stream segment.

Material and Methods

Study area and collection methods

Sampled streams are located in the study areas of the Biological Dynamics of Forest Fragments Project (BDFFP, INPA/Smithsonian Institute). The BDFFP is situated about 80 km north of Manaus (20°25'S, 60°W) and encompasses approximately 3,500 ha of forest made up of 1, 10, and 100 ha forest fragments, as well as large extensions of continuous forest (Gascon & Bierregaard, 2001). These areas are classified as humid tropical forest with mean annual precipitation of 1900-2500 mm, a rainy season from December to May, and a dry season from June until November. Vegetation coverage has an average height of 30-37 m, with emergent trees reaching 45-50 m and an understory with abundant palms (Gascon & Bierregaard, 2001).

We sampled three streams in the Cabo Frio, Fazenda Dimona, and Km 41 BDFFP study sites. The streams are connected to basins of the Preto da Eva (Cabo Frio) and Urubu (Km 41) rivers, both tributaries of the Amazon River, and to Cuieiras River (Fazenda Dimona), a tributary of the Negro River. Samplings were conducted between May and August 2004, which corresponds to the early dry season in the central Amazonia. At each locality, one 1st order, one 2nd order, and one 3rd order stream segment was sampled a single time. Stream order was determined *in loco* according to Horton's scale modified by Strahler (Petts, 1994), where the junction of two first order streams form a second order stream and two second order streams form a third order stream. Table 1 shows the geographic coordinates for sampled streams. In this study, we refer to streams by their locality, Cabo Frio (CF), Dimona (DI), or Km 41 (QU), and segment order, 1st, 2nd, or 3rd. All streams are located in continuous forest, with the exception of DI 1st order segment, which is partially located in a forest fragment of 100 ha. Distances between segments of each stream (1st to 3rd) ranged 0.35-3.17 km, and between 14.3 and 37.3 km among different streams. Stream substrate was composed mainly of coarse litter and sand patches, with a few submerged trunks and tree branches. In some reaches of 3rd order segments there were some bog plants (*Thurnia sphaerocephala*, Thurniaceae) partially covering the stream bottom.

Table 1. Geographic localization and mean values (minimum - maximum) of environmental parameters of 100-m stream segments of 1st, 2nd, and 3rd order located in the study areas of Biological Dynamics of Forest Fragments Project, Manaus, Amazonas State.

Order	Stream	Geographic coordinates	Width (m)	Depth (cm)	Volume (m^3)
1st	CF	S2°24'28", W59°54'58"	1.58 (1.15-2.25)	13.88 (8-24.1)	21.52
	DI	S2°20'45", W60°05'34"	1.12 (0.74-1.4)	7.98 (4.4-12.9)	9.05
	QU	S2°26'34", W59°46'41"	1.27 (0.78-1.7)	10.38 (3.8-17)	12.94
2nd	CF	S2°24'45", W59°54'19"	1.99 (1.5-2.6)	21.84 (15.7-29)	43.31
	DI	S2°21'00", W60°05'49"	2.83 (2.32-3.62)	33.93 (25.7-45.5)	96.32
	QU	S2°26'26", W59°46'28"	1.65 (1.18-2.1)	14.07 (5.9-19.1)	23.25
3rd	CF	S2°24'54", W59°54'12"	3.78 (2.5-4.92)	57.27 (47.3-67.4)	217.18
	DI	S2°19'45", W60°04'39"	4.23 (3.77-5.19)	54.54 (41.3-66.9)	229.51
	QU	S2°25'56", W59°46'17"	2.73 (2.1-3.25)	40 (21.67-75.9)	110.17

Fish sampling

Our sampling design consisted of five 20-m reaches sampled in each stream segment, in three segments (1st, 2nd, and 3rd order) of three streams (Cabo Frio, Dimona, and Camp 41). We randomly chose five 20-m reaches along each stream segment and each reach was separated by a 15-m section of stream. Prior to the samplings the ends of each 20-m reach were blocked with fine meshed (5 mm stretched mesh) nets to avoid fish escapes. We sampled the five reaches in the upstream direction to minimize effects of drifting materials and other disturbances on the other stream reaches. We employed hand nets, metallic sieves (50 cm diameter, 3.5 mm mesh), and small fine-meshed seine nets to collect fishes. All fish sampling took place during the morning and early afternoon. Collecting effort at each reach was conducted by the same team in all streams, and consisted of the activity of three people during 45 min in 1st order stream segments, three persons and 60 minutes in 2nd order stream segments, and four persons and 60 minutes in 3rd order stream segments. Collecting at each stream segment was completed on the same day and each reach sample was preserved in separate containers for analysis. Fish samples were preserved in 10% formalin and vouchers were deposited in the Fish Collection of the National Institute for Amazonian Research in Manaus (INPA 25308 to INPA 25496).

Stream dimensions and physical variables

In order to explore relationships between stream size and fish species density, we measured stream width (m) and depth (cm) across the streambed, in 10-cm intervals beginning 10 cm from the stream margin. Each set of measurements was taken at two points within each 20-m stream reach. To estimate the volume of water (m^3) in each stream reach, we used the measured values of length, width, and depth, and assumed a trapezoidal form for the channel section. These variables are represented by mean values along the text.

Data analysis

We recorded species density (number of species) and abundance (number of individuals) for each stream reach. To evaluate differences among reaches within each stream segment and among stream segments of the same order, we used the mean ($\overline{X}$), standard deviation (sd), minimum (min), maximum (max), and range values of species density and fish abundance. We used the Jaccard's coefficient (Krebs, 1989) to estimate the similarity of species composition among stream reaches and segments. Subsequently we pooled the samples of the five 20-m reaches of each segment (then representing a 100-m segment) and generated estimates of species richness with Jackknife 1 and Bootstrap algorithms using the software EstimateS 7.5 (Colwell, 2005). Then we compared these results with the actual accumulated species density values of each 100-m stream segment.

To obtain the asymptote for the species accumulation curve in each segment, we generated average values of the number of accumulated species in function of the random addition of samples (five 20-m stream reaches), adjusted to a growth function type: $y = a(b\text{-}e^{-cx})$ using the software Curve Expert 1.3 (Hyams, 2001). These values (hereafter referred to as Sass) were also used as species richness estimates. Based on asymptote values and species accumulation curves equations, we then estimated the mean minimum sampling length that best estimates the absolute number of species in a stream segment.

We used simple linear regression (Zar, 1996) to look for relationships between species density and fish abundance and between species density and the volume (pooled for the five, 20-m reaches) of each segment. Before these analyses, we tested for homogeneity of variances, normality, and linearity of the data, assumptions for the application of regression (Zar, 1996).

Results

Studied streams segments were generally narrow, with mean width varying between 1.12 and 4.23 m ($\overline{X} = 2.35 \pm 1.11$ sd), and mean depth varying between 7.98 and 57.27 cm ($\overline{X} = 28.20 \pm 18.97$ sd). The volume of the pooled five 20-m reaches of each segment varied widely from 9.05 to 229.51 m^3 ($\overline{X} = 84.81 \pm 86.35$ sd) (Table 1).

Overall, 61 species and 2200 individuals were caught in the nine surveyed stream segments. Captures per 20-m reach varied in both species density and fish abundance. These variations occurred both between reaches within the same segment and within streams segments of the same order.

Species density values did not exceed a difference of six species between reaches of each segment, and nine species among reaches of segments of the same order. There was a slightly higher variation between 1st order reaches, whereas fish abundance was apparently higher in 3rd order reaches (as shown by sd values in Table 2). Similarity in fish species composition among 20-m reaches was low for all studied streams, but 3rd order segments showed slightly lower values when compared with 1st and 2nd order segments (as demonstrated by mean values in Table 3). Overall fish assemblage similarity among segments of Cabo Frio and Km 41 streams varied between 0.66 and 0.68, whereas similarity values involving Dimona stream segments were somewhat lower and ranged 0.55-0.59.

Species accumulation curves based on extrapolations of our samples (n=5) showed an increase in the number of species with the number of reaches sampled, but did not reach an asymptote, even after the fifth reach was added (Fig. 1). Nevertheless, we observed that segments located in Cabo Frio and Km 41 streams tended to have more similar species accumulation values, when compared to segments of the Fazenda Dimona stream. We found that accumulation curves for DI 1st order reaches leveled at lower richness values than for CF and QU reaches. The opposite trait was observed for 2nd and 3rd order reaches, in which the DI curves leveled at higher values, with higher species richness than the CF and QU curves.

Table 2. Fish abundance and species density in 20-m reaches of 1st, 2nd, and 3rd order streams segments located in the study areas of Biological Dynamics of Forest Fragments Project, Manaus, Amazonas State. Total= pooled values for five 20-m stream reaches. General= mean values by stream order.

Order	Stream	Species density						Abundance					
		mean	sd	min	max	range	total	mean	sd	min	max	range	total
1st	CF	7.2	2.2	4	10	6	12	23.8	8.3	14	37	23	119
	DI	3.8	1.3	2	5	3	8	43.8	10.9	36	63	27	219
	QU	8.2	1.6	7	11	4	14	57.0	10.9	44	74	30	285
	General	6.4	2.5	2	11	9	20	41.5	16.9	14	74	60	623
2nd	CF	9.4	2.1	6	11	5	16	30.8	8.4	22	42	20	154
	DI	11	1.2	9	12	3	22	53.0	15.5	35	75	40	265
	QU	7.8	2.4	5	11	6	15	34.8	10.9	16	44	28	174
	General	9.4	2.2	5	12	7	34	39.5	14.9	16	75	59	593
3rd	CF	11.8	2.4	9	15	6	25	51.0	19.4	23	77	54	255
	DI	21.0	3.8	17	25	8	42	102.4	35.1	59	154	95	512
	QU	12.6	2.3	9	15	6	24	43.4	22.3	21	71	50	217
	General	15.1	5.1	9	25	16	58	65.6	36.6	21	154	133	984

Species richness values observed for accumulated captures in five 20-m reaches (corresponding to a 100-m stream segment) represented 71.4% to 94.1% of the estimated number of species for these stream segments. Values calculated with Bootstrap and species accumulation curves were similar to each other and lower than those generated by the Jackknife 1 method (Table 4). We estimate that the mean minimum sampling length to best estimate the absolute number of species in a stream segment is 180 m ± 20 sd for a 1st order segment; 213 m ± 23 sd for a 2nd order segment, and 253 m ± 30 sd for a 3rd order stream segment (Table 5, Fig. 1).

Abundance data showed homogeneity of variances (Levene's= $F_{(2,6)} = 2.85$; p= 0.134), and species density data were logarithmically transformed to fit this assumption (Levene's= $F_{(2,6)} = 0.53$; p= 0.61). Species density showed a direct relationship both with stream segment volume ($F_{(1,7)} = 25.76$; $r^2 = 0.79$; p = 0.001; n = 9; Fig. 2a) and abundance of individuals ($F_{(1,7)} = 6.86$; $r^2 = 0.5$; p = 0.03; n = 9; Fig. 2b).

Table 3. Similarity values (Jaccard's coefficient) of species composition among samples of 20-m reaches of 1st, 2nd, and 3rd order stream segments located in the study areas of Biological Dynamics of Forest Fragments Project, Manaus, Amazonas State. General= mean values by stream order.

Order	Stream	Similarity				
		mean	sd	min	max	range
1st	CF	0.54	0.17	0.36	0.87	0.51
	DI	0.45	0.16	0.29	0.80	0.51
	QU	0.57	0.14	0.38	0.78	0.20
	General	0.32	0.17	0.08	0.87	0.79
2nd	CF	0.57	0.10	0.43	0.75	0.32
	DI	0.47	0.09	0.33	0.60	0.27
	QU	0.46	0.10	0.33	0.63	0.30
	General	0.30	0.19	0	0.75	0.75
3rd	CF	0.41	0.09	0.29	0.58	0.29
	DI	0.42	0.05	0.35	0.51	0.16
	QU	0.44	0.09	0.32	0.65	0.33
	General	0.24	0.14	0.03	0.65	0.62

Discussion

Species richness has been used as an important measure of biodiversity, for both defining conservation strategies and comparative ecological analyses (Wilson, 1997; Primack & Rodrigues, 2002; Melo, 2003; Santos, 2003). However, species richness and abundance values, attributes typically used to characterize the structure of fish communities, are known to be very sensitive to sampling effort. Angermeier & Smogor (1995) observed discontinuities in the distribution of fish species within and among streams, and found that such discontinuities bear strongly on the sampling effort that is necessary for a characterization of the community structure. As such, the choice of a sample size that permits the generation of reliable estimates of these attributes is a central question in studies of ecological communities (Cao *et al*., 2002).

Table 4. Species richness in 100-m segments of 1st, 2nd, and 3rd order streams, estimated by the methods of Jackknife 1, Bootstrap and by extrapolation from species accumulation curves. Cl= confidence limits of methods of Jackknife 1; Sobs= observed richness; Sass= richness estimated by extrapolation from species accumulation curves; %Jack= percent of observed species richness in relation to that estimated by the Jackknife method; %Boots= percent of observed species richness in relation to that estimated by the Bootstrap method; %Sass= percent of observed species richness in relation to that estimated by extrapolation from species accumulation curves.

	Streams								
	CF	DI	QU	CF	DI	QU	CF	DI	QU
Order	1st	1st	1st	2nd	2nd	2nd	3rd	3rd	3rd
Sobs	12	8	14	16	22	15	25	42	24
Jackknife	14	10	18	21	30	19	35	54	30
(Cl)	(±2)	(±4)	(±7)	(±6)	(±0)	(±6)	(±6)	(±7)	(±4)
Bootstrap	13	9	16	18	26	17	29	48	27
Sass	13	10	16	18	27	17	31	48	27
%Jack	85.7	80	77.8	76.2	73.3	78.9	71.4	77.8	80
%Boots	92.3	88.9	87.5	94.1	84.6	88.2	86.2	87.5	88.9
%Sass	92.3	80	87.5	94.1	81.5	88.2	80.7	87.5	88.9

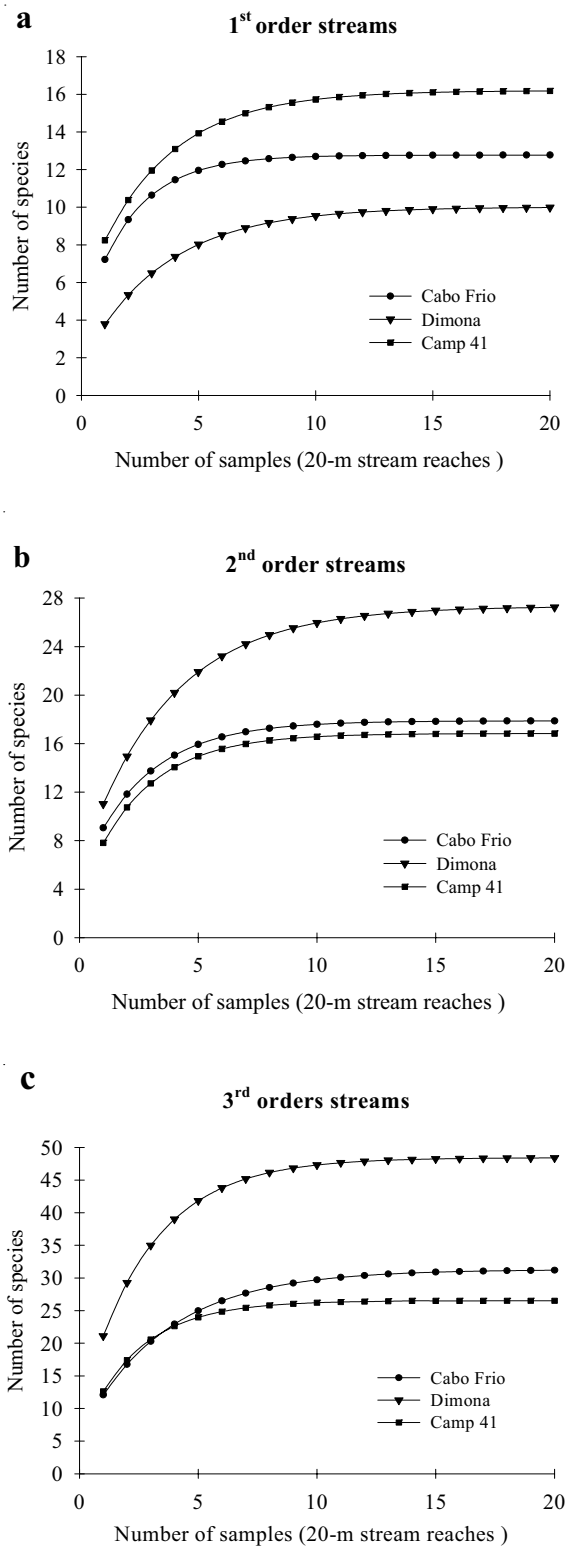

Fig. 1. Fish species accumulation curves estimated from samples obtained in 1st, 2nd, and 3rd order streams reaches located in the study areas of Biological Dynamics of Forest Fragments Project, Manaus, Amazonas State. The curves represent extrapolations from five reaches sampled in each stream segment.

Table 5. Summary of equations of species accumulation curves, linear regression parameters, asymptote values (= estimated number of species) and length of stream segment estimated to result in a complete survey of species at each site. x= number of samples (20-m reaches) necessary to the curve to reach an asymptote.

Order	streams	Equation	R	asymptote	x (samples)
1st	CF 1	$y=8.96(1.42-e^{-0.48x})$	0.999	13	8 (160 m)
	DI 1	$y=8.27(1.21-e^{-0.28x})$	0.999	10	10 (200 m)
	QU 1	$y=10.9(1.48-e^{-0.31x})$	0.999	16	9 (180m)
2nd	CF 2	$y=12.9(1.39-e^{-0.38x})$	0.999	18	10 (200 m)
	DI 2	$y=21.5(1.27-e^{-0.28x})$	0.999	27	12 (240 m)
	QU 2	$y=13.3(1.26-e^{-0.39x})$	0.999	17	10 (200 m)
3rd	CF 3	$y=25.4(1.23-e^{-0.28x})$	0.999	31	13 (260 m)
	DI 3	$y=38.9(1.24-e^{-0.35x})$	0.999	48	11 (220 m)
	QU 3	$y=21.2(1.25-e^{-0.42x})$	0.999	27	14 (280 m)

If a sample actually represents the community from which it was collected, it is expected that subsequent samples collected under the same conditions will produce similar values of species density and composition. In the present study, data obtained for the 20-m reaches of the nine stream segments were extremely variable with respect to composition and species density. This suggests that estimates based on samples of this size may not provide a good enough representation of those fish communities. We do not believe that the high variability in species density and assemblage com-

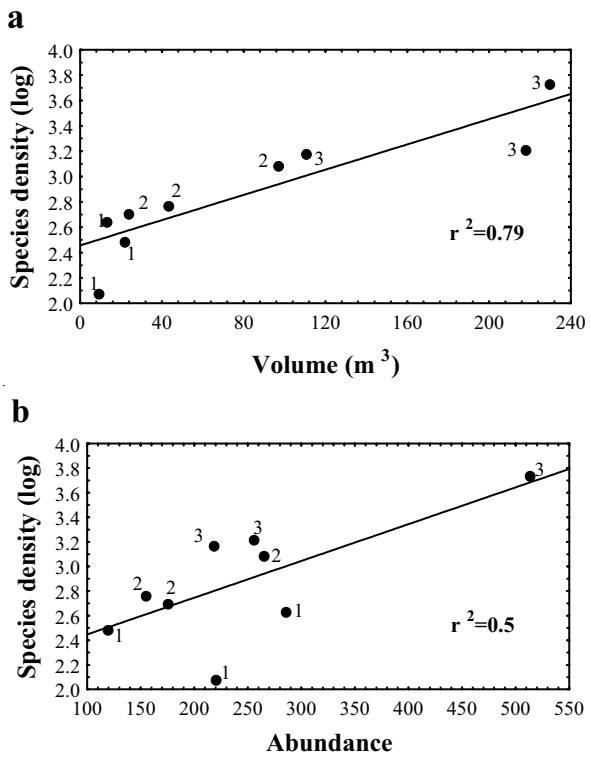

Fig. 2. Relation between fish species density (a), fish abundance (b) and water volume (m^3) in 100-m stream segments of 1st, 2nd, and 3rd order located in the study areas of Biological Dynamics of Forest Fragments Project, Manaus, Amazonas State. The numbers 1 to 3 refer to the stream order.

position observed in our study result from inadequate sampling effort, since our sampling protocol was established based on a large series of samples obtained in several locations in Brazilian Amazon (unpublished data). The low abundance of fish in 20-m reaches also represents a common feature of central Amazonian streams. Furthermore, the very low catches observed at the end of each sampling period indicates a near exhaustion of fishes in each stream reach and support the adequacy of our sampling procedure. A possible disturbance effect generated by the collecting team along the stream segment from first to last reach may also be discarded, since there wasn't any obvious tendency in similarity values obtained along each set of five reaches sampled (data not shown). Finally, the small time interval between samplings (three months) arguably rules out the possible effect of seasonal differences among fish assemblages sampled in the three streams, since fish assemblage composition vary little in small-order forest streams in the central Brazilian Amazon (Lowe-McConnell, 1991; Buhrnheim & Cox-Fernandes, 2001).

Habitat heterogeneity is known to have a strong effect on the distribution of fish species along streams, both in function of major longitudinal features (e. g. the sequence of riffles and pools) and microhabitat diversity (Angermeier & Karr, 1984; Garutti, 1988; Sabino & Castro, 1990; Taylor, 2000; Buhrnheim, 2002). Mérigoux *et al*. (1998) showed that fish species richness and composition along coastal streams in French Guiana were related to habitat diversity. Araújo-Lima *et al.* (1999) studied the fish fauna in streams at one of our study sites (Km 41) and also found a direct relation between fish species richness and habitat complexity. In the present study, the sampling points were chosen without previous knowledge about habitat heterogeneity (*i.e.* microhabitat diversity) within each stream reach, which may explain (at least in part) the differences observed in species density among reaches and segments. In fact, the lowest species richness was observed in the 1st order stream segment at Fazenda Dimona, which was the smallest of all studied segments and also had a remarkably homogeneous substrate formed mostly by coarse litter.

The relation between sampling effort and species richness (as shown by species accumulation curves) was steeper in larger stream segments, possibly due to species-area relationships and also environmental heterogeneity (Harrel *et al*., 1967; Gorman & Karr, 1984; Angermeier & Schlosser, 1989). A given length of a higher-order stream segment will have a larger flooded area and a larger volume of water than a similar length of a lower-order stream segment. The observed variations in fish species richness of same-order stream segments in the present study were partially explained by the volume of water in the segment. The 2nd and 3rd order stream segments at Fazenda Dimona are larger (and seemingly more structurally complex) than similar-order segments in the other two sites, which might explain the higher species richness observed in that stream. It seems plausible that larger streams contain higher number of microhabitat types, which allow for the presence of more fish species per stream length. Thus, a larger stream length makes it possible to survey a wider variety of habitats and consequently, to collect rare species that occupy uncommon microhabitats, increasing the species richness recorded. For example, Zuanon *et al.* (2006) showed that there are several sand-dwelling fish species with very patchy distributions along streams. So, the eventual absence of such habitat in a given stream reach or segment would result in the absence of a whole subset of the fish assemblage in that stream section, generating low similarity values among reaches or segments.

Although part of the variation in observed fish species density can be explained by differences in stream dimensions and habitat diversity, we cannot rule out the possible effects of stream connectivity to different basins. The streams located in the Cabo Frio and Km 41 sites are connected by higher-order tributaries to the Amazon River, whereas the Fazenda Dimona stream connects through the Cuieiras River to the Negro River. Mendonça *et al.* (2005) found some differences in fish assemblages between streams connected to these two major river basins and argued that low connectivity between the two basins and very different water characteristics (pH, suspended solids, and other chemical differences) of the Negro and Amazon rivers may represent an effective barrier to some fish species. This low connectivity (and possible effects of an ecological barrier) may explain part of the low similarity observed between stream segments in the present study, although historical factors can't be ruled out.

Studying fish assemblages of small temperate and tropical streams, Angermeier & Schlosser (1989) concluded that the number of individuals is often the best predictor of species richness in a sample. Although we observed this trend in our study, the best predictor of species richness was stream segment volume. However, the low number of streams surveyed and presence of outliers (e.g. the Dimona 1st order stream) may also have had an effect on our results.

The extrapolation of species accumulation curves generated reliable estimates of fish species richness in sampled streams. Such supposed reliability is based on the similarity of values obtained with the Bootstrap and Jackknife methods (in fact, a little smaller but within the confidence limits of the Jackknife estimates). It is also known that Jackknife estimates are based on the presence of rare species and tend to overestimate richness when the number of samples is small (Krebs, 1989; Santos, 2003).

According to our findings (and adopting a conservative approach), the fish fauna in these stream segments could be confidently characterized if we use the higher values of stream length estimated for each stream order, that is, 200-m for 1st order, 240-m for 2nd order, and 280-m for 3rd order. Lyons (1992) sampled 10 warm-water streams in southern Wisconsin using a towed electrofishing unit and also found that the recommended length of stream to sample for confidently estimating species richness could be an absolute distance specified in meters. Nevertheless, species richness is often related to the number of habitat units sampled, which varies in function of

stream size (Simonson *et al*., 1994). Sampling relatively long stream reaches makes it more likely that a sample may include a wide variety of microhabitats and thus reduces the possible effects of different distribution patterns among fish species in the samples. However, we should emphasize that the use of a variety of sampling devices and techniques, including gill nets and fike net traps, especially in larger streams, can add consistency to the protocol we propose here.

The application of the proposed sampling protocol in a variety of habitats and at a wider regional scale would provide important information about its validity for Amazonian *terra firme* forest streams in general. Such data would generate information about the variation of fish species diversity and provide reference values that could be used in the characterization of the Amazonian ichthyofauna and definition of fine-tuned regional strategies for biological conservation.

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