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Chapter 7

Diversity and Abundance of Fish Larvae Drifting in the Madeira River, Amazon Basin: Sampling Methods Comparison

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Additional information is available at the end of the chapter

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1. Introduction

The Amazon River and its main tributaries are known as downstream migratory way of eggs and larvae of many migratory fishes [1, 2, 3]. The young fish drift may occur during all year but the great abundance is associated with the flood pulse pattern, generally with the rising of the river level [4, 5]. The eggs, larvae and juveniles may drift for hundred or thousand kilometers and repopulate the stocks in the down river portion [6; 7; 8; 9].

The maintaining of the larval drift process is fundamental for the conservation of a great number of migratory fishes as well as for the maintaining of local economy and food security. The migratory species are the most important fisheries resource of the Amazon Basin and they are responsible for about 87% of the total landing in 66 ports of the main Amazon cities [10]. The fishery production, in special the fishery of migratory species, maintains an important sector of the Amazon economy, generating about US$130 million yr^{-1} and more than 160 thousand jobs [11]. The fish consumption rate by Brazilian Amazonian population is 24 kg annual per capita or 343 thousand ton/year, almost four times of the fish consumption rate of the Brazilian population [12].

One of the biggest threats to the drift movement of young fish drift movement are large dams, which interrupt the river connection and reduce or change the natural flood pulse pattern [13, 14]. There were few large hydroelectric dams in the Amazon basin, but this situation is changing by the construction of several hydroelectric dams in large tributaries of the Amazon River [15]. The most recent dams are two hydroelectric power plants nearby Porto Velho city, in the State of Rondônia, Brazil, which are damming the largest tributary of the Amazon River, the Madeira River.

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The Madeira River Basin occupies 1.4 million km^2 or 20% of the Amazon Basin, which 50% belongs to Bolivia, 40% to Brazil and 10% to Peru. The Andes headwater is in Bolivia and Peru and the lowland portion and the Brazilian Shields headwater are in Brazil. The Madeira River at the frontier border of Bolivia and Brazil is characterized by a sequence of waterfalls, which separate the Llanos de Mojo savanna in Bolivia, the largest floodplains area of the Madeira River, and its lowland portion [16]. The Jirau and Santo Antonio hydroelectric power plants are being built above of the low portion in order to generate energy from the waterfalls of the Madeira River. The reservoirs of both dams should occupy an area around 530 km^2, which will flood only the Brazilian territory. The highest difference between the upper and lower limits of both reservoirs is about 50 m (Environmental Impact Assessment (EIA) of Madeira River, http://www.ibama.gov.br/licenciamento/index.php).

In order to assess the impact of the dams over the ichthyoplankton drifting along the Madeira River, it was established by the hydroelectric company a sampling protocol to collect periodically along the river before closuring the dam and, then in the reservoir, just below the dam. One methodology adopted was used to compare the larval density estimated from samples taken at different depths along the river transect [2, 3, 4, 5, 23]. In addition, it was developed a methodology in order to estimate the larval flux in different cross section of the river. This methodology was adapted from the direct methods for measuring suspended-sediment discharge in rivers [25]. The utilization of both methods will help to decrease the gear selectivity of the nets and methodologies and to compare the results of each one.

The aim of this study is to compare the selectivity of three sampling methods over the species composition and developmental stage of fish, as well as characterize the drift movement of ichthyoplankton in the waterfalls stretch of the Madeira River, considering the flood seasonal and spatial variation, before the closure of the Jirau Dam. The present study is a reference to the future impact assessment of the ichthyoplankton drift pattern related to the establishment of power plants.

2. Study area

The fish eggs, larvae and juveniles were collected monthly from October 2009 to September 2012 in two sampling sites in the Madeira River. The locations of those sites were defined by the limits of the planned reservoir of the Jirau Hydroelectric Power Plant (HPP). The sample sites were established in the upper and downriver limits of the planned reservoir in order to compare future modifications of the drift fish larvae patterns. At upriver site is the confluence of Abunã and Madeira River (9º 40′ S-65º 26′W), and at downriver site is the Jirau Hydroelectric Power Plant (9º 15′ S-64º 38′W). Between these two sampling sites, there are four perennial rapids (Pederneiras or Tamborete, Paredão, Jirau and Caldeirão do Inferno) and five seasonal rapids (Machado, Prainha, Vai Quem Quer, Dois Irmãos and Embaúba). Along this stretch there is the biggest rapid, known as Jirau waterfall, which is located about 13 km upside of Jirau HPP (Figure 1).

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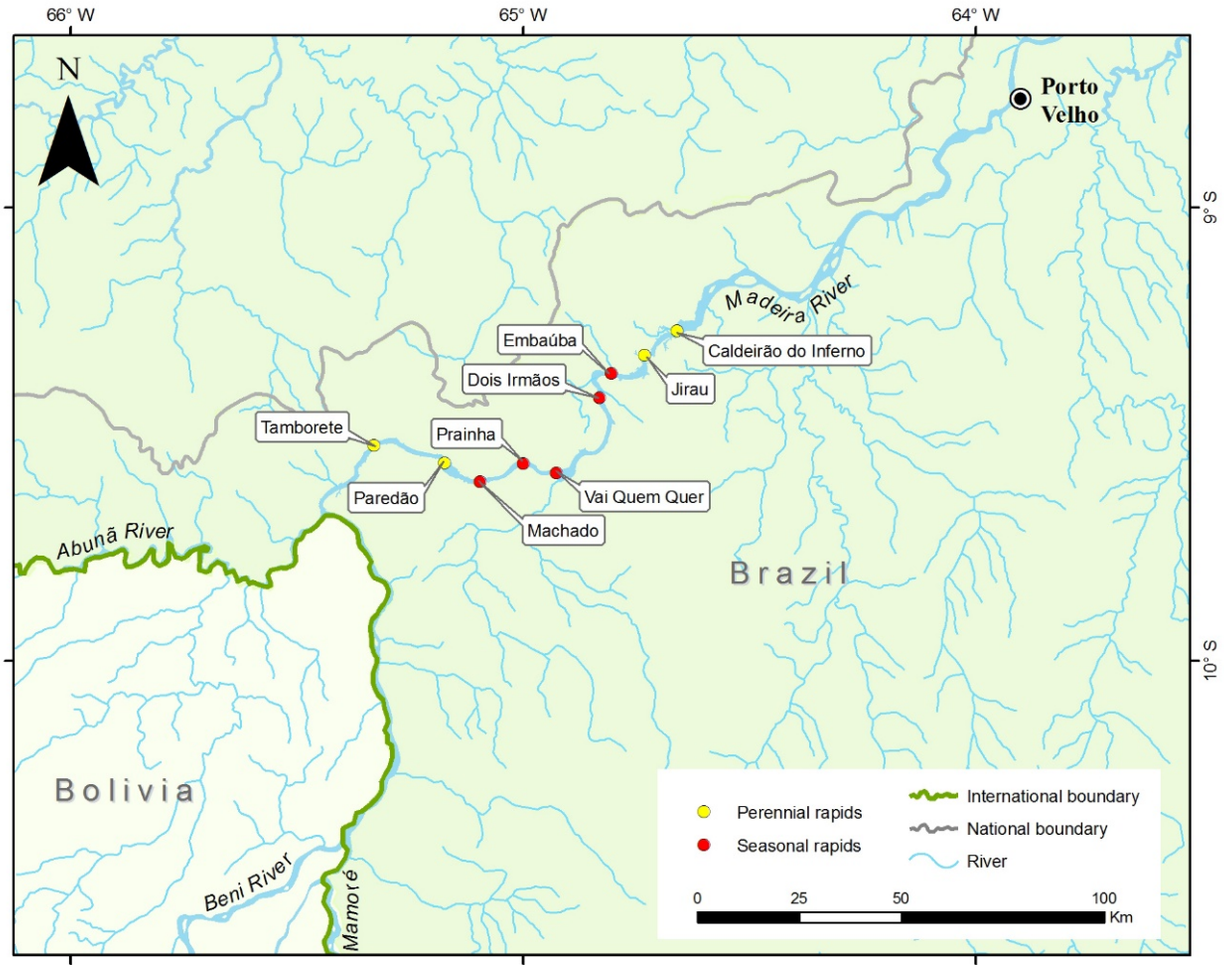


Figure 1. Perennial and seasonal rapids between the two sampling sites in the Madeira River.

The river's length between two sampling sites is about 140 km, river's width ranges from 300 to 1200 m, and altitude varies from 72 to 92 m. The flooding area in this stretch is very narrow if compared to the flooding area of the Beni or Mamoré River in upriver of the Jirau´s Reservoir. The annual precipitation is about 2,650 mm, with intensive rainfall from December to April, while the driest period goes from May to November. The difference between high and low water level, in general, goes from 10 to 12 m [16]. The monthly mean of freshwater discharge ranges from 34,512 m^3/s, in March, and 5,959 m^3/s, in September, showing an annual mean of 19,222 m^3/s (River Discharge Database-Sage: http://www.sage.wisc.edu).

3. Sampling and data analysis

Ichthyoplankton samples were obtained in two perpendicular transects in the Madeira River located at two sampling sites. In the Abunã site, at upriver, was established one transect above and other below of the confluence with the Abunã River and Madeira River. At downriver

site, the positions of transects were established upside and downside of the Caldeirão do Inferno rapid, where the Jirau Hydroeletric Power Plant (Jirau HPP) is being built (Figure 2).

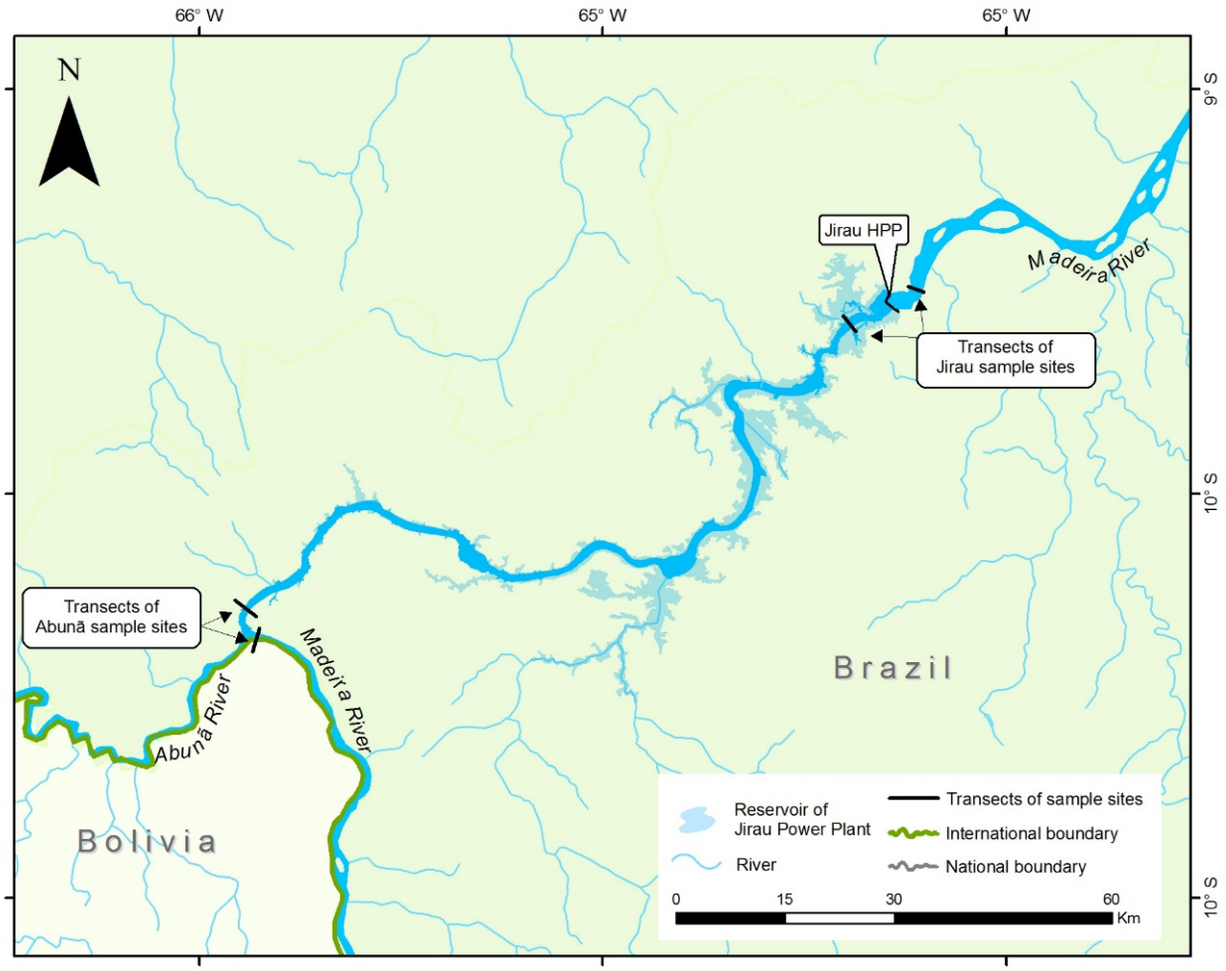


Figure 2. Four transects where ichthyoplankton samples are collected in reservoir area of the Jirau HPP, in Madeira River Basin.

The quantitative samples of ichthyoplankton were obtained during the daylight by towing an ichthyoplankton net from an outboard motor boat equipped with an electric winch. During sampling, the boat's position was maintained by the motor, which was moving upstream with the same speed of the river. Nocturnal samples were not collected because was assumed that the ichthyoplankton drift does not vary along day periods, as reported for rivers that show similar physicochemical characteristics of Madeira River [2].

Two different nets were used to collect ichthyoplankton in the Madeira River. The first one, nominated as larval net, has a conical-cylindrical format, with an aperture diameter of 0.5 m (0.1935 m^2), 1.5 m length, and a mesh size of 0.5 mm [17]. This type of net has a collector cup used to open and close the net at different depth. The second one, nominated as juvenile net, was developed to collect large larvae and juvenile fish. It has a square frame in the open mouth,

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with 1 m^2 of area, 2 m of length, and 5 mm of mesh size. The volume of water filtered was measured by a flowmeter set up in the mouth of each net.

It was tested two different sampling methods to estimate the abundance of the ichthyoplankton drift in the Madeira River. The first used the point sampling method [18] and fixed five sites sampling in a linear transect to take samples in margins, main channel and zones in-between the main channel and the margins [3, 5]. The second used the integrating sampling method [18], which applied the systematic sampling method to define the sites sampling along of transect. The Point sampling with Larval net - PL - collects two samples in different depth at the water column. The remote open/close system set up in the mouth of the larval net ensured the net to collect samples at one meter from surface and at 70% of the total depth. The time spent in each sample was five minutes, and the distance from the margin ranged from 5 to 20 m. The Integrating sampling using Larval net – IL - and Juvenile net – IJ - takes one integrating samples of the water column, from the surface to the bottom and back. The velocity of net moving down and up was constant and determined by an electric winch. The distance of sampling sites was about 100 m each other and, at least, 20 m of the margin. The number of spots in each transect was related with width of the river.

The physical and chemical characteristics of the Madeira River's water were monitored by measuring dissolved oxygen (mg/l), pH, conductivity (μS/cm), temperature (°C) and turbidity (ntu) in the surface and at 70% of the bottom depth of each sample spot. The estimative of the freshwater discharge of the Madeira River was based on the river level of the Abunã site. The river level and the river discharge of the Abunã station was supplied by fluviometric station located in the district of Abunã, in the city of Porto Velho / RO (UTM 20L 240534 and 8926519), operated by the Geological Survey of Brazil (CPRM).

The ichthyoplankton samples were fixed in a 4% formalin solution and then they were counted and identified considering the taxonomy and the developmental stages. The developmental stages considered in this study were egg, larva yolk, pre-flexion, flexion, post-flexion and juvenile [19, 20]. The fish larvae were identified according to [21, 17].

The larval density was determined for each sample considering the number of ichthyoplankton and the volume of water filtered (larvae/m^3). The larval flux (larvae/s) of the cross section of the river was estimated multiplying the average of the larval density of the transect by the diary discharge (m^3/s). It was not considered the discharge of the small tributaries along the study area due to this minimal contribution to the overall freshwater discharge. The larval flux variation was analyzed by Factorial ANOVA, which tested the effect of the sampling method, river site and ichthyoplankton development stage. Pearson coefficient (r) was used to test the relationship between the variables.

4. Hydrology and environment variables

The freshwater discharge of the Madeira River is characterized by an annual unimodal cycle defined by four phases: low, rising, high and falling water level. The low water level phase is

when the discharge is minimal and the river beach is exposed, usually between August and November, while the high water level phase is when the discharge is high and the river floods the marginal areas, it occurs generally between February and May. Other months are considered transition phases when the water level is rising or falling. The rising water level phase is when begins the rainy season and the river discharge starts to increase, between December and January, and the falling water level phase is when the river discharge decreases and the flood retreats, between June and July. The diary discharge median estimated for the study period was 15 thousand m^3/s, which was used to separate the low and high discharge phases. Interannual variations of the hydrological cycle were observed during the period studied, with a short and intensive high discharge phase in 2010 (<160 days and >39 thousands m^3/s) in relation to other years (>190 days and <36 thousands m^3/s) (Figure 3).

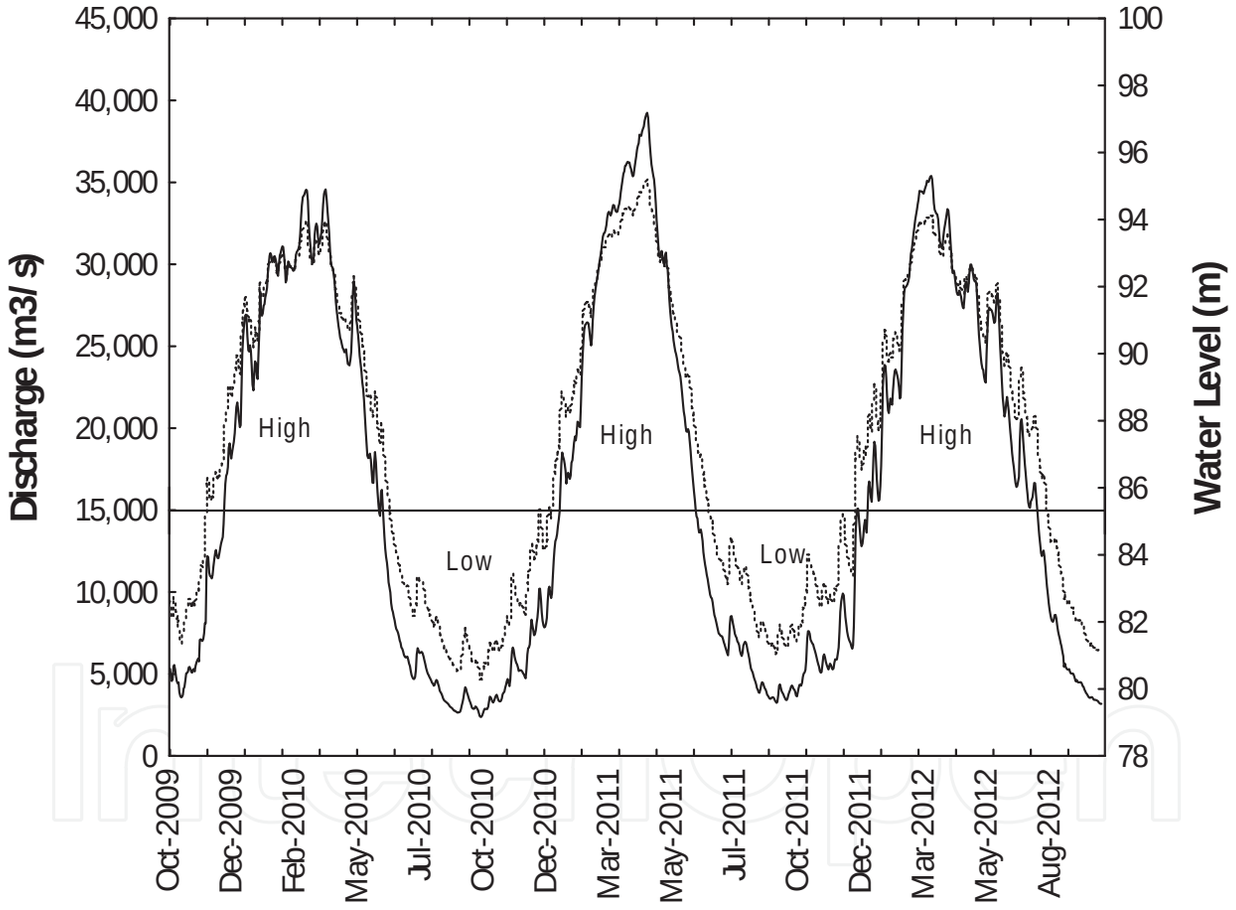


Figure 3. The hydrological cycle of the Madeira River Basin obtained at the Hydrological Station of Abunã, at the up-river of the study area (discharge- continuous line; water level- dotted line).

The Madeira River is a muddy river that receives a large amount of sediments rich in mineral salt, which comes from Andean region. Due to this, the monthly averages of the conductivity and pH are in general higher than the other rivers of the Amazon Basin, varying from 69 to 131 μS/cm and 6 and 8, respectively. The conductivity and pH values were inversely related

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to the discharge, and the highest values occurred during the low discharge period ($r_{conductivity}$= -0.74, r_{pH}= -0.46; p<0.01, n=36). The mean turbidity is also high in relation to the other rivers, varying from 84 to more than one thousand NTU, with the highest turbidity value occurring in the beginning of the high discharge period (Pearson correlations: r= 0.63, p<0.01, n=36). The dissolved oxygen mean was relatively high, which can be associated with the aeration process caused by the movement of water in the rapids. The monthly average ranged from 5.7 to 8.8 mg/l and there was an inverse relationship with the discharge (r= -0.41, p<0.05, n=36). The high river discharge is associated with the flooding of the wetland and decomposition of large amount of organic material, which consumes oxygen of the aquatic environment (Table 1).

Month	Conductivity	Temperature	Oxygen	Ph	Turbidity
1	73	27.5	8.6	7.03	1.137
2	69	27.1	7.0	6.83	1.129
3	73	27.0	5.7	6.94	961
4	78	27.6	6.7	7.10	564
5	80	27.6	6.7	6.69	401
6	74	26.9	7.6	6.75	298
7	90	27.2	8.9	6.08	205
8	107	26.4	8.8	7.88	180
9	131	28.1	8.6	8.07	84
10	121	29.4	7.8	7.98	411
11	105	29.4	7.5	7.48	320
12	78	27.9	7.1	7.28	806
Mean	89.61	27.67	7.59	7.17	551.57
SD	22.68	1.14	2.20	0.87	421.58

Table 1. Average monthly of the physicochemical parameters obtained in the Madeira River.

5. Ichthyoplankton abundance

During the three years, 4,148 individuals were collected by 432 samples of ichthyoplankton realized monthly in four transects, with an average of 9.5 samples by transect and method. The number of samples were similar for each combination of sampling methods and nets, however there were more samples in downriver (Jirau: 56%) than in upriver (Abunã: 44%). This difference in samples number was due to the river width, which is related to the number of sampling sites for integrating sampling method. A total of 21,665 larvae (99%) and 282 eggs (1%) were collected. The point sampling (PL) method was more efficient at collecting eggs than the integrating sampling methods. The PL collected 53% of the eggs, followed by the integrating sampling method with juvenile net (IJ), that collected 29%, and the integrating sampling method with larvae net (IL), that caught the remains 17% of the eggs. The PL method also

collected more larvae (61%) than the other methods and the IL method collected more larvae (34%) than the IJ method (5%). The number of eggs was similar for the both sites, and in the upriver (Abunã) showed twofold more larvae than in downriver (Jirau), even at downriver showing more samples (Table 2).

Sites	S&N	Eggs			Larvae & Juveniles		
Upriver (Abunã)	IJ	53	19%	49%	631	3%	66%
	IL	19	7%		4,316	20%	
	PL	65	23%		9,369	43%	
Downriver (Jirau)	IJ	30	11%	51%	489	2%	34%
	IL	30	11%		2,979	14%	
	PL	85	30%		3,881	18%	
Total		282	100%		21,665	100%	

Table 2. Number of eggs, larvae, juveniles collected in up, downriver places, and the combination of sampling methods and nets: IJ: Integrating sampling with juvenile net; IL: integrating sampling with larval net; and PL: point sampling with larval net.

The development stage dominant in all samples was pre-flexion (66%), followed by flexion (19%) and post-flexion (5%). This stage composition was similar for the both sites, but different for the net types, where more than 95% of the ichthyoplankton caught by IL and PL were in flexion or early stages and 99% of the ichthyoplankton caught by IJ was in flexion or older stages. Considering each development stage, about 2/3 of all larvae in larval yolk or pre-flexion stages were caught by the PL method, whilst 49% of all larvae in post-flexion stages and 71% of juveniles were caught by the IJ method. Only nine small fish in adult stage were collected during this study (Table 3).

Stage	Methods And Nets			Sites		Total	
	IJ	IL	PL	Upriver	Downriver		
Unknown	22	403	318	423	320	743	3%
Larval Yolk	7	376	790	910	263	1,173	5%
Pre-Flexion,	7	4,599	9,713	9,830	4,489	141319	66%
Flexion	396	1,667	2,073	2,368	1,768	4,136	19%
Post-Flexion	522	220	319	647	414	1,061	5%
Juvenile	159	30	35	134	90	224	1%
Adult	7	0	2	4	5	9	0%
Total	1,120	7,295	13,250	14,316	7,349	21,665	100%

Table 3. Composition of the larvae collected considering the development stages and in relation to the up and downriver places and the combination of sampling methods and nets (IJ: integrating sampling with juvenile net; IL: integrating sampling with larval net; and PL: point sampling with larval net).

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The abundance index considered the average of the larval density and the estimative of the larval flux in the cross section of four transects along the Madeira River. Peaks of larval density and flux were observed during the rising discharge and the beginning of the high discharge phases (December to March) and a decreasing in the next months. However, larval flux was minimal from June to November while larval density showed some peaks during this period

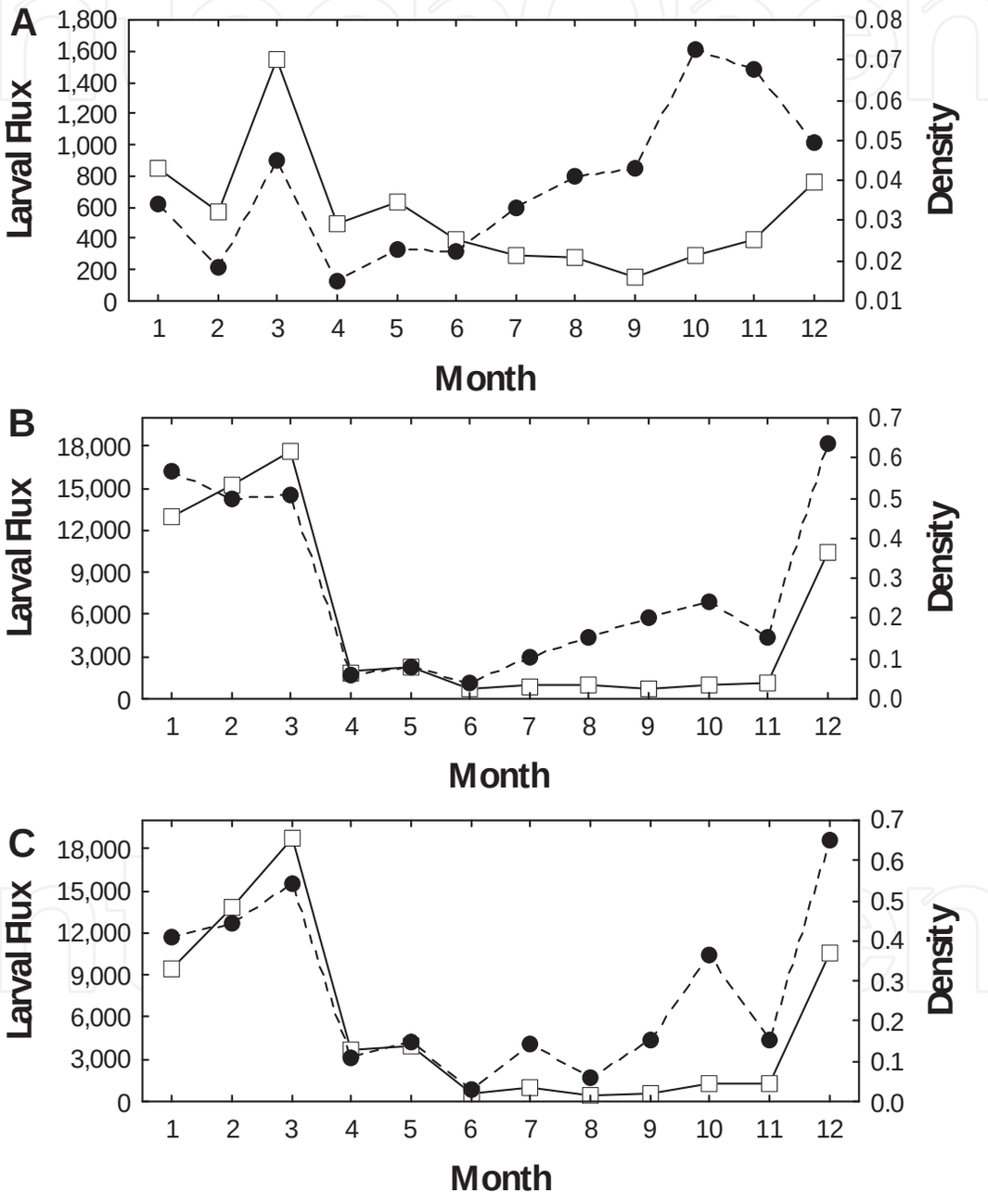


Figure 4. Monthly variation average of the larval flux (larvae/s) (□) and larval density (larvae/m³)(●) estimated for (A) integrating sampling with juvenile net-IJ; (B) integrating sampling with larval net-IL and (C) point sampling with larval net-PL. Months: 2-5 (February-May) high discharge; 6-7 (June-July) falling discharge; 8-11 (August-November) Low discharge; 12-1 (December-January) rising discharge.

(Figure 4). The relationship between the two abundance indexes for the three methods is presented in Figure 5. The highest correlation value between larval density and flux was observed for the IL method (r^2=0.86) and the lowest values was observed for the IJ method (r^2=0.23). The correlation between IL and PL methods was higher for larval flux (r^2=0.77) than for larval density (r^2=0.61) (Figure 5). The mean composition of the larval flux by larval stages of 144 samples shows the importance of IJ and IL methods for the juvenile abundance estimative and the IL and PL for the abundance estimative of the early stages (Table 4).

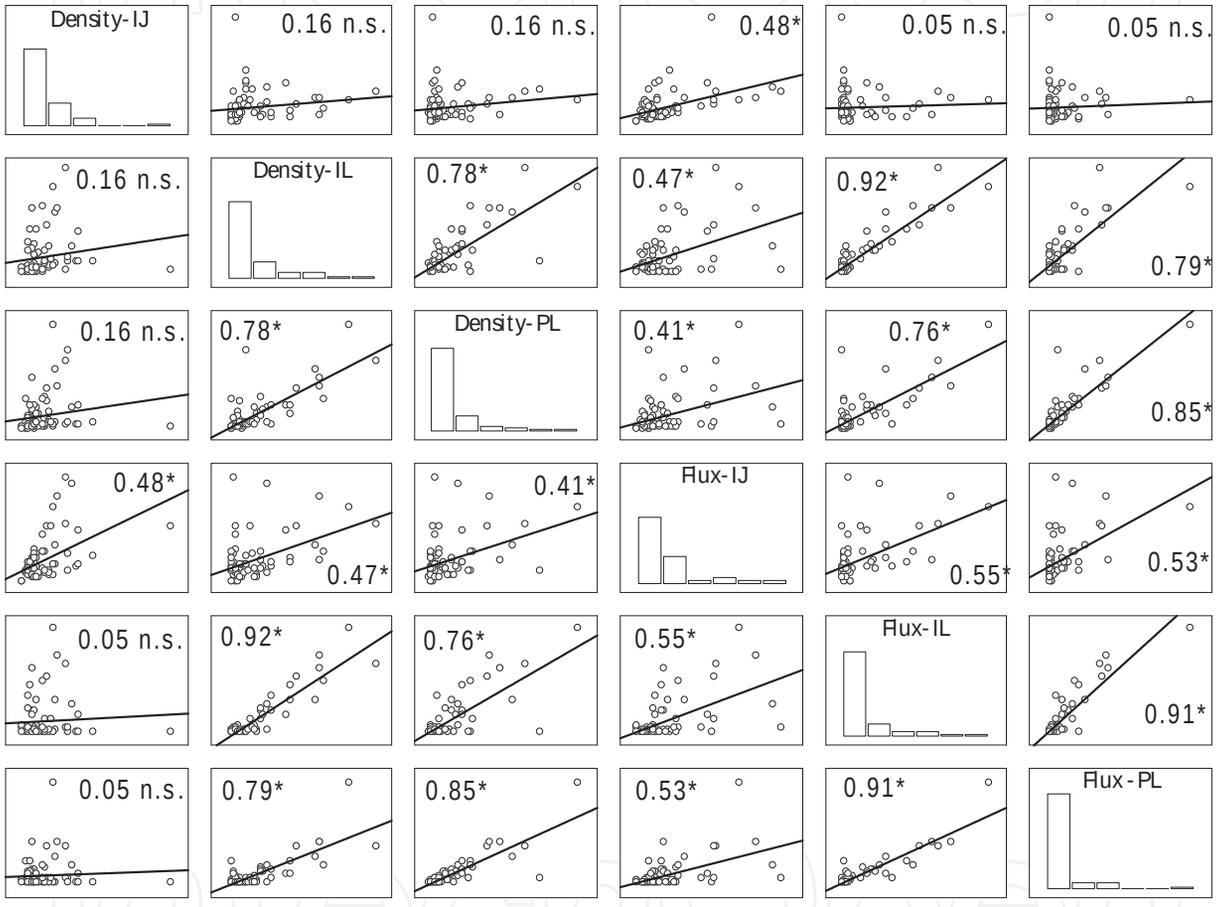


Figure 5. Abundance indexes plots for the three sampling methods and the respective Person's coefficient *r* (* p<0.01, n=144; n.s: no significant). Abundance indexes: Density (larvae/m^3) and Flux (larvae/s). Methods: IJ: integrating sampling with juvenile net; IL: integrating sampling with larval net; and PL: point sampling with larval net.

The seasonal and spatial variation of the larval flux was analyzed only for IL and PL data. The IJ data was not considered due its low capacity to detected larvae in early stages and to assure the assumptions for homogeneity of variance. An ANOVA-two-way was performed to analyze the variation in larval composition considering the square root of the larval flux as dependent variable and the sampling method (IL and PL), the up and downriver sites and five development stages (larval yolk, pre-flexion, flexion, post-flexion and juvenile) as independent variables (Table 5). The assumptions for homogeneity of variance was met according to the

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Stage	IJ	IL	PL	Total
Larval Yolk	12 (1%)	685 (55%)	539 (44%)	1,236 (100%)
Pre-Flexion	13 (0.2)%	3,675 (48%)	3,996 (52%)	7,684 (100%)
Flexion	217 (7%)	1,507 (52%)	1,175 (41%)	2,898 (100%)
Post-Flexion	344 (19%)	677 (38%)	758 (43%)	1,779 (100%)
Juvenile	198 (41%)	201 (42%)	82 (17%)	481 (100%)
Total	822 (5%)	7,261 (48%)	6,944 (46%)	15,027 (100%)

Table 4. Average and percent of the development stage composition for each method considering larval flux (larvae/s). Methods: IJ- integrating sampling with juvenile net; IL- integrating sampling with larval net; and PL-point sampling with larval net.

Levene's test ($F=0.31$, $p>0.05$). The larval flux estimated by PL method is significantly lower than the IL method for all stages (Method: $F=7.53$, $p<0.01$), especially for juvenile stage, but the interactive effects of sampling method with the other factors was not significant (Method-Site, Method-Stage, Method-Site-Stage: $p>0.05$). The larvae of the pre-flexion stage were more abundant than the other stages (Stage: $F=26.10$, $p<0.01$). The up and down river sites was not a significant factor (Site: $p>0.05$), but the interactive effect with development stage was significant (Site-Stage: $p<0.05$) indicating larvae in pre-flexion stage is higher in upriver than downriver, but the other stages presented similar values in both sites (Tukey HSD test, $p<0.01$) (Table 5).

	SS	*d.f.*	*MS*	*F*	*p*
Intercept	580306.0	1	580306.0	689.5715	<0.0001**
Method	6340.9	1	6340.9	7.5349	0.0062**
Site	1882.5	1	1882.5	2.2369	0.1352
Stage	87861.8	4	21965.5	26.1013	<0.0001**
Method-Site	23.4	1	23.4	0.0278	0.8676
Method-Stage	891.3	4	222.8	0.2648	0.9006
Site-Stage	8308.0	4	2077.0	2.4681	0.0436*
Method-Site-Stage	2193.1	4	548.3	0.6515	0.6260
Error	637050.2	757	841.5		

Table 5. Result of Factorial ANOVA testing the methods (IL and PL), river site (up and downriver) and development stage (larval yolk, pre-flexion, flexion, post-flexion and juvenile) factors over the square root of the larval flux. S.S. = sum of squares; d.f. = degrees of freedom; M.S. = mean square; F = F statistic; p = significance level. One asterisk means significant at 0.05 level and two asterisks means significant at 0.01.

6. Larval diversity

Eggs and larvae identification depends on the integrity of the sampled larvae as well as on previous ontogeny studies. High diversity of the Amazon fish and the paucity of ichthyoplankton ontogeny studies become the larvae identification a challenge. Despite of this, just 0.3% of the larvae and juveniles collected were completely unknown and only 8% of them were identified at the order level, the lowest possible level. The most larvae (92%) was identified at family level and the identification at genus and species level was possible in 30% and 13% of the total larvae, respectively. The Characiformes and Siluriformes larvae were dominant in the samples and represented about 98% of the total larvae collected. The PL net method collected almost twofold more Characiformes than Siluriformes larvae, while IJ method collected almost fourfold Siluriformes than Characiformes larvae. The larvae composition collected by IL method did not show a large discrepancy among the most important taxonomic groups, even so Characiformes were more abundant than Siluriformes larvae (Table 6).

Curimatidae, Auchenipteridae and Pimelodidae were the most abundant families that totalizing about 2/3 of the total larvae. The Curimatidae and Auchenipteridae families represented the half of the larvae collected by the PL method and the Pimelodidae family represented 2/3 of the collected by IJ method. The caught of three families aforementioned were similar for the IL method. The Characidae family was the fourth in the rank of abundance, representing about 12% of the larval composition collected by the three methods. The larvae of Prochilodontidae, Anostomidae and Hemiodontidae together represented 12% of the total larvae. The sampling methods using larval net collected 95% or more of the larvae of Characidae, Prochilodontidae, Anostomidae and Hemiodontidae families, which they represented together about ¾ of all larvae caught. Other families represented less than 5% of the total (Table 6).

Less than 5% of the larvae of Curimatidae, Auchenipteridae, Anostomidae, Prochilodontidae and Hemiodontidae families, the most abundant families, were identified at the genus level. Nonetheless, 94% of the Characidae larvae and 83% of the Pimelodidae were identified at the genus level. The larvae of *Brycon*, *Mylossoma*, *Triportheus* and *Piaractus* genera were the most abundant among the Characidae family. Each one represented more than 5% of all Characidae larvae and together represented 93% of the Characidae larvae and 11% of all larvae, but few of them were identified at the species level. On the other hand, most larvae of the four most abundant genera of the Pimelodidae family were identified at the species level. *Pinirampus*, *Brachyplatystoma*, *Pimelodus* and *Pseudoplatystoma* represented each one more than 5% of all Pimelodidae larvae and together represented 71% of Pimelodidae larvae and 13% of all larvae.

The Curimatidae, Auchenipteridae and Anostomidae families are diversified and they were represented in the study area by 7 genera and 21 species, 18 genera and 21 species and 6 genera and 13 species, respectively. For the other side, the diversity of Prochilodontidae and Hemiodontidae families are low and they have only two genera with three species and two genera with six species in the area, respectively [22].

[22] identified three species of *Brycon* (*B. amazonicus, B. falcatus* and *B. melanopterus*), two species of *Mylossoma* (*M. aureum* and *M. duriventre*) and three species of *Triportheus* (*T. albus, T.*

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	Order	Total		IJ		IL		PL	
Order	Unknown	63	0,3%	0	0%	29	0.4%	34	0.3%
	Characiformes	12,485	58%	231	2%	4,005	55%	8,249	62%
	Siluriformes	8,806	41%	825	74%	3,182	44%	4,799	36%
	Perciformes	189	1%	20	2%	49	1%	120	1%
	Clupeiformes	69	0%	12	1%	21	0.3%	36	0.3%
	Gymnotiformes	53	0%	32	3%	9	0.1%	12	0.1%
Family	Unknown	1,834	8%	27	2%	832	11%	975	7%
	Curimatidae	5,891	27%	9	1%	1,676	23%	4,206	32%
	Auchenipteridae	3,965	18%	11	1%	1,272	17%	2,682	20%
	Pimelodidae	3,960	18%	717	64%	1,582	22%	1,661	13%
	Characidae	2,650	12%	134	12%	880	12%	1,636	12%
	Prochilodontidae	1,482	7%	1	0,1%	495	7%	986	7%
	Anostomidae	749	3%	1	0,1%	231	3%	517	4%
	Hemiodontidae	276	1%		<0.1%	101	1%	175	1%
	Cynodontidae	251	1%	71	6%	70	1%	110	1%
	Sciaenidae	188	1%	20	2%	48	1%	120	1%
	Doradidae	116	1%	67	6%	20	0.3%	29	0.2%
	Trichomycteridae	92	0.4%	5	0.4%	34	0.5%	53	0.4%
	Cetopsidae	61	0.3%	3	0.3%	16	0.2%	42	0.3%
	Heptapteridae	45	0.2%	3	0.3%	16	0.2%	26	0.2%
	Apteronotidae	27	0.1%	22	2%	2	<0.1	3	<0.1
	Callichthyidae	15	0.1%	34	1%	2	<0.1	5	<0.1
	Loricariidae	15	0.1%	16	0.1%	7	0.1%	7	0.1%
	Engraulidae	13	0.1%	9	1%	3	<0.1	1	<0.1
	Pristigasteridae	11	0.1%	3	0.3%	2	<0.1	6	<0.1
	Erythrinidae	8	<0.1	2	0.3%	4	0.1%	2	<0.1
	Gasteropelecidae	8	<0.1	0	<0.1	2	<0.1	6	<0.1
	Sternopygidae	7	<0.1	6	1%	0	0%	1	<0.1
	Clupeidae	1	<0.1	0	0%	0	0%	1	<0.1
	Total	21,665	1	1,120	100%	7,295	100%	13,250	100%

Table 6. Larvae composition caught by the combination of methods and nets: IJ- integrating sampling with juvenile net; IL- integrating sampling with larval net; and PL- point sampling with larval net.

angulatus and *T. auritus*) in the study area. The present study identified the two species of *Mylossoma* and only two species of Triportheus (*T. angulatus* and *T. auritus*) and none of *Brycon*. Considering the shortage of larvae identified at specie level, it is not possible to discuss the composition of those genera. On the other side, all *Pinirampus* larvae and 99% of the *Brachyplatystoma* larvae were identified at specie level. *Pinirampus pirinampu* was the only specie of the genus. Five *Brachyplatystoma* species were identified, being *B. filamentosum, B. rousseauxii* and *B. capapretum* the most abundant, totalizing 90% of the genus. The other two species in the larval collection were *B. platynemum* and *B. juruense*. Two *Brachyplatystoma* species, *B. tigrinum* and *B. vaillantii*, were present in the local fish collection [22] but they are not present in the present larvae samples. Nevertheless, only 56% of the *Pimelodus* and 11% of the *Pseudoplatystoma* genus were identified at specie level. *Pimelodus blochii* was the main specie identified of the genus and only one individual represented *P. altissimus* was in all samples. The two species of *Pseudoplatystoma* present in the area were in the larvae samples, *P. punctifer* and *P. tigrinum*, but most larvae were not identified at specie level. The larval net caught the majority of the larvae of those species, with the exceptions of *P. blochii, B. rousseauxii* and *B. capapretum*, which the juvenile net caught more than 50% of the larvae of each specie.

The majority (c.a. 95%) of the larvae of the most families were in pre-flexion or earlier stages, whilst Characidae and Pimelodidae presented the larvae in advanced stage. The most Characidae larvae were found in pre-flexion (63%) and flexion (35%) stages, while Pimelodidae larvae were found in flexion (69%) and post-flexion (23%) stages. However, the development stage composition was different for each species or genus. *Mylossoma* spp., *Triportheus* spp. and *Piaractus brachypomus* presented 90% or more of the larvae in pre-flexion stage, while *Brycon* spp. showed developed larvae, with 87% in flexion stage. The majority (2/3 or more) of *Pseudoplatystoma* spp., *Zungaro zungaro, Pinirampus pirinampu*, *Brachyplatystoma filamentosum* and *B. capapretum* larvae were in flexion stage, and more than a half of *Pimelodus blochii* and *B. rousseauxii* were in post-flexion stage.

The larvae drift in the river occurred mainly when the discharge is increasing. Some families concentrated the drifting movement in just a few months and others remained drifting during all year, indicating the length of the reproductive season. The average larval flux estimated by the three samples method in the two sites was compared in order to understand the general drifting pattern of the most abundant taxa. The IL and PL methods were efficient to identify the great variation of the larval flux for most of the families and IJ detected satisfactorily the annual flux variation only for Characidae and Pimelodidae.

The Curimatidae and Prochilodontidae families showed the shortest reproductive season between December and April, but strongly concentrated in March. The larvae of Auchenipteridae and Anostomidae families remained drifting in the river for a longer period, between November and May, with peak in January and March. The general pattern of the larval drifting of the Characidae and Pimelodidae families is also intensive in the period of rising discharge and less intensive in the low discharge months. However, the results of the three methods showed some differences, especially in April and May, when the IL method pointed the end of reproduction season and the PL method still detects an intensive flux of larvae (Figure 6). *Brycon* spp. and *Piaractus brachypomus* showed a shorter spawning season, during the rising

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discharge months (December-January), and *Mylossoma* spp. extended the reproduction beyond the period of the rising discharge (December-March). *Triportheus* spp. presented a delayed drifting larval movement in comparison with the other species. The peak was detected in March by the IL method and in May by the PL method, both moments in the high discharge period (Figure 7).

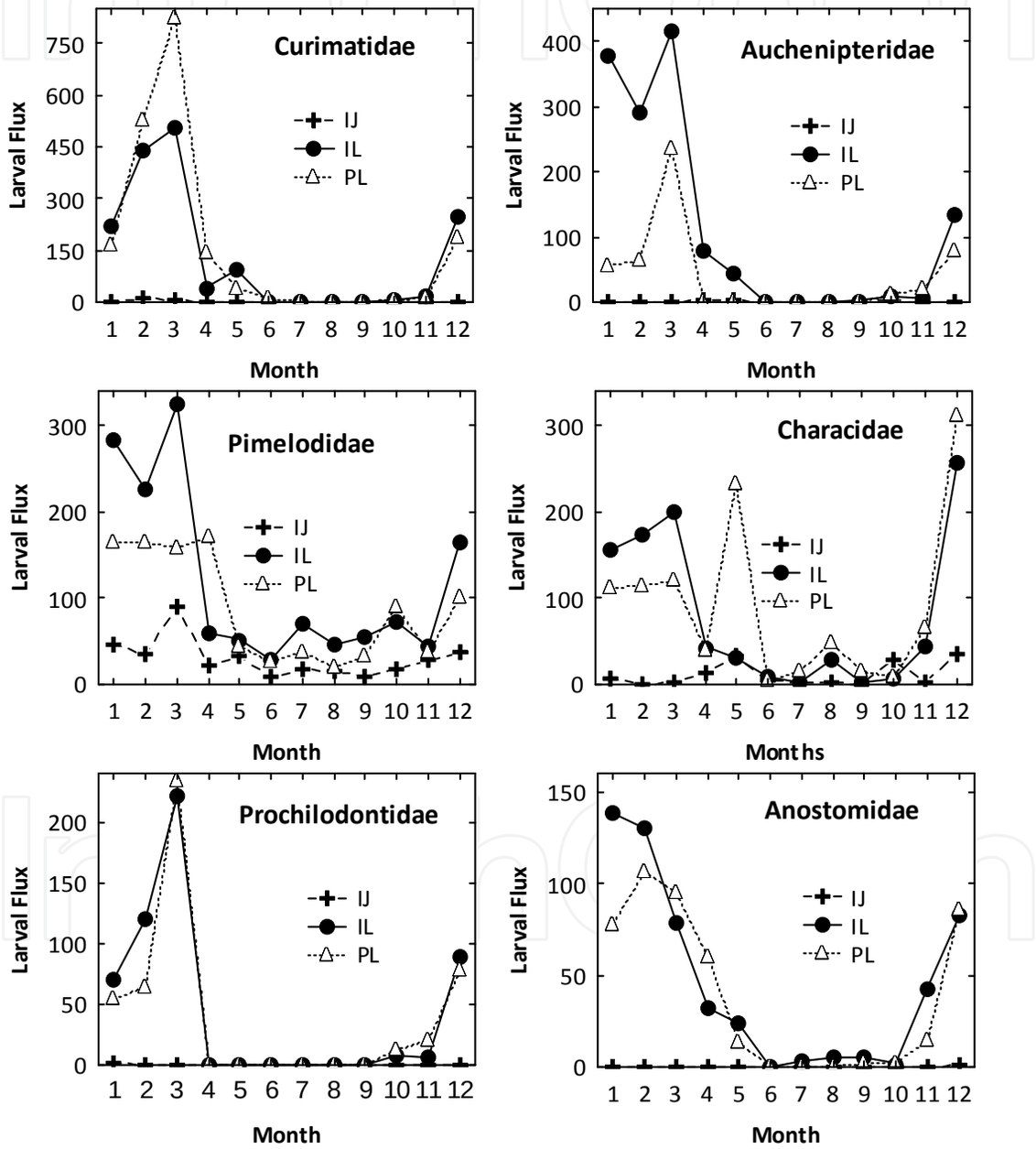


Figure 6. Monthly average of the larval flux (larvae/s) of main families: IJ- integrating sampling with juvenile net; IL- integrating sampling with larval net; and PL- point sampling with larval net. Months: 2-5 (February-May) high discharge; 6-7 (June-July) falling discharge; 8-11 (August-November) low discharge; 12-1 (December-January) rising discharge.

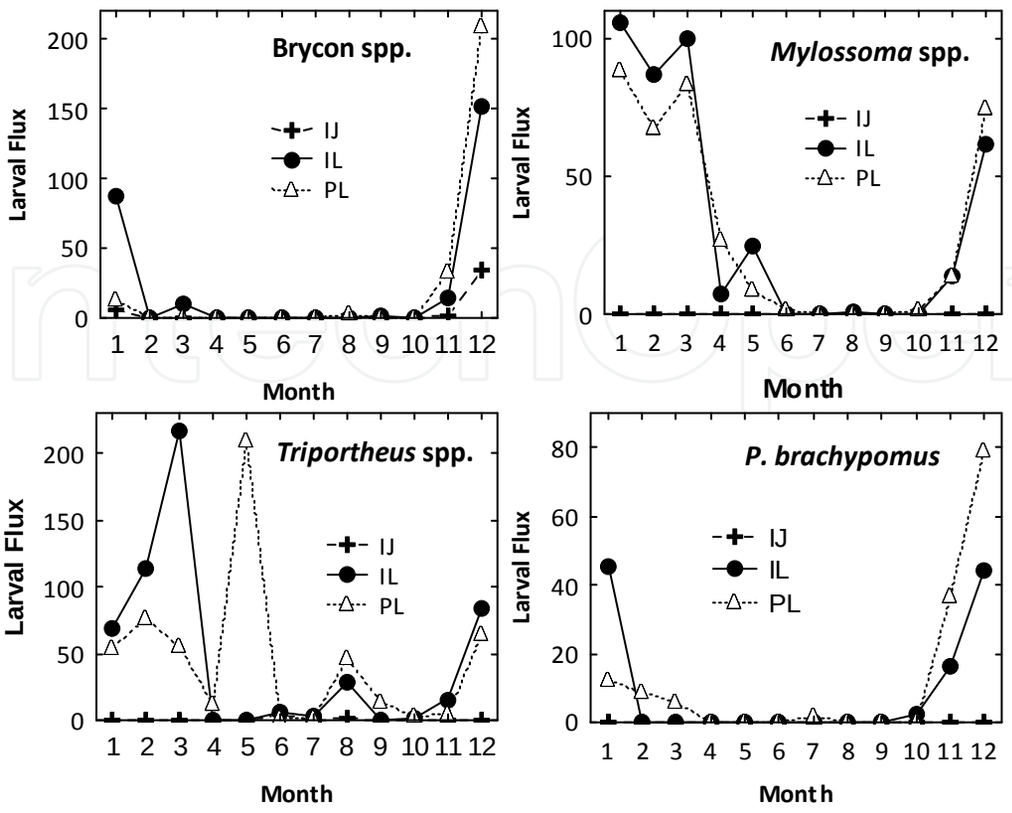


Figure 7. Monthly average of the larval flux (larvae/s) of the main species of Characidae family: IJ- integrating sampling with juvenile net; IL- integrating sampling with larval net; and PL- point sampling with larval net. Months: 2-5 (February-May) high discharge; 6-7 (June-July) falling discharge; 8-11 (August-November) low discharge; 12-1 (December-January) rising discharge.

The drifting movement of the most abundant Pimelodidae species was longer than the Characidae species. In addition, the IJ method presented an expressive flux estimative when compared with the other groups, in special for the *Brachyplatystoma rousseauxii* and *Pimelodus blochii*. Despite of large amount of larvae of each species, drifting in the channel during the rising and or high discharge months, the movement of larvae did not stop during the low discharge period suggesting an uninterrupted spawning season. Larvae of *Pseudoplatystom*a spp. and *P. blochii* were scarce during the falling and low discharge, but the larvae of *Pinirampus pirinampu* or *Brachyplatystoma* spp. were abundant in that period. The different sampling methods also presented expressive differences in the larval flux of some Pimelodidae species. *P. blochii, B. capapretum* and *B. rousseauxii* presented isolated peaks of larval flux detected by only one method (Figure 6).

7. Discussion

The point sampling method carried out at fixed habitats along of transects is a good way to compare the larval abundance or composition in different depths or habitats [2, 3, 4, 5, 23]. However, when the aim is to assess the impact of the river modification over the ichthyo-

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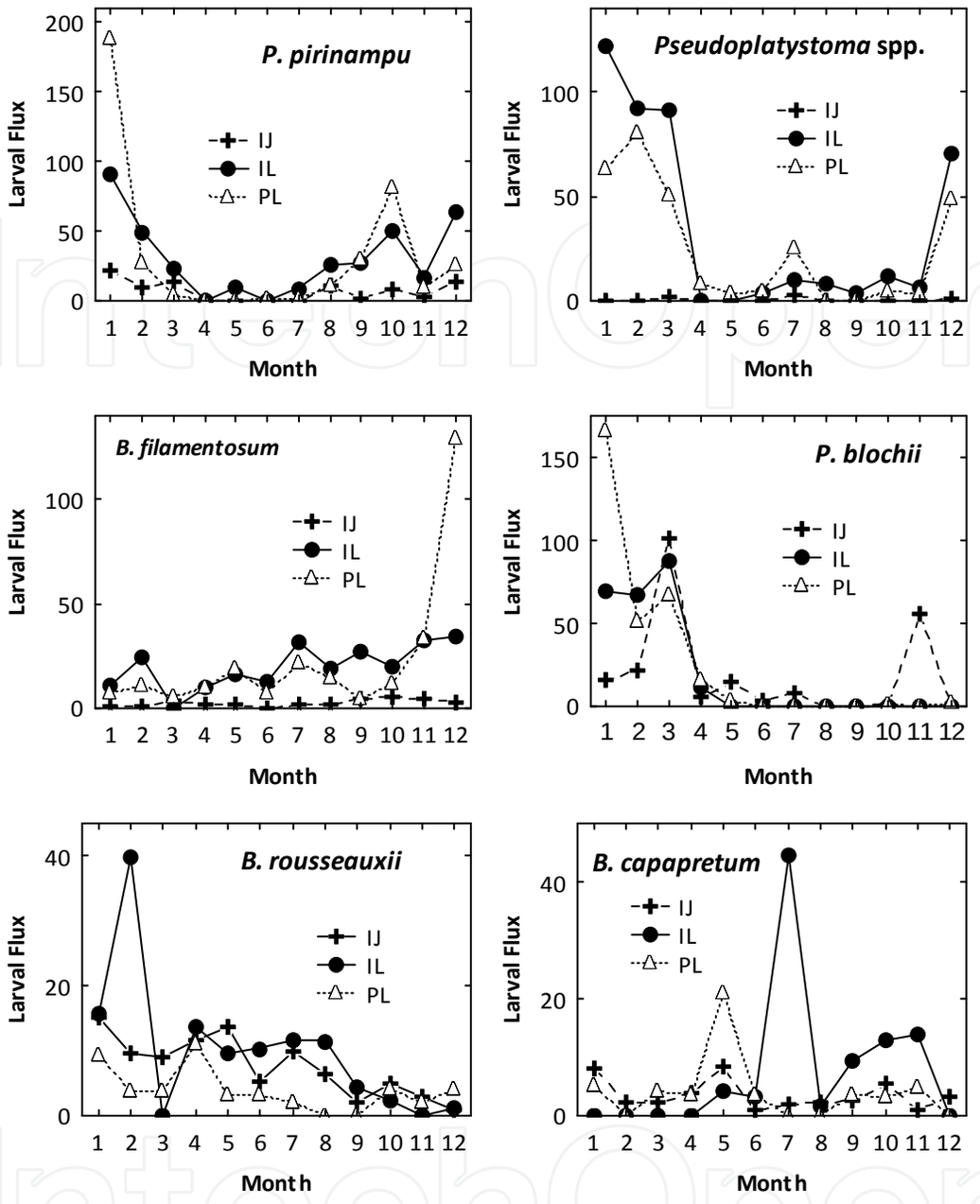


Figure 8. Monthly average of the larval flux (larvae/s) of the main species of Pimelodidae family: IJ- integrating sampling with juvenile net; IL- integrating sampling with larval net; and PL- point sampling with larval net. Months: 2-5 (February-May) high discharge; 6-7 (June-July) falling discharge; 8-11 (August-November) low discharge; 12-1 (December-January) rising discharge.

plankton drifting process, it is necessary to develop a methodology adapted to estimate the larval flux in natural or dammed river cross sections.

The three methods caught few eggs in relation to total of ichthyoplankton, and it was similar for the up and downriver sites. [4] also found few eggs in the ichthyoplankton composition in the Solimões River (Table 2). They hypothesized that the eggs have a short residence time, less than 16 hours, and as the spawning occurs mainly during the dusk, the eggs had hatched before

the sampling moment. The point sampling and the larval net caught more individuals than the integrating sampling and the juvenile net. However, the juvenile net caught mainly larvae in post-flexion stages and juveniles when compared to samples collected by larval net (Table 3 and 4). Using juvenile net is appropriated to sample ichthyoplankton of species that go through the study area in advanced development stages, like *Pimelodus blochii* and *B. rousseauxii*.

The abundance indexes, larval density and flux considered in this study, pointed an increasing of larvae and juveniles abundance during the rising discharge and the beginning of the high discharge phases (December to March), and a decreasing in the next months. It was also observed by [4], in Solimões River, and by [3], in the Madre de Dios River. However, the larval flux index was minimal from June to November while larval density index presented some expressive peaks during May to November. The relationship of both indexes was low, although had been statistically significant, for the IJ method and high for the IL and PL methods (Figure 5). The larval density is affected by the dilution effect, which occurs during the rising and high discharge, and by the concentrating effect, that occurs during the low water discharge. In that way, the larval density is a measure more accurate for the drift inversely proportional to the water discharge if the larval flux is constant. The larval flux is a more accurate measure to assess the changes of the larval drift in modified rivers. This measure is not biased by the discharge moment or by the width or depth of the river cross section.

The composition of the larval flux in relation to the larval development stages of each method (Table 4) indicated the IL method as the less selective in relation to the development stages of the fish when they are drifting in the river. The IJ method is selective for juveniles and underestimated the abundance of all larvae in early phases and the PL method is selective for larvae and underestimated the abundance of juvenile phase.

The larval flux estimated by the IL method was higher than the larval flux estimated by the PL method and they were similar in the upper and downriver sites, in spite of the number of larvae caught in the upriver was two times of the number caught downriver (Table 2 and 5). The two methods estimated the larval flux in pre-flexion stage as bigger than the larval flux in other stages and the larval flux in pre-flexion stage were more abundant in upriver than downriver, while the larval flux of the other stages were similar in both sites (Table 5). The upriver abundance of larvae in early stages may suggest a more intensive spawning activity more intensive upriver of the study area.

The identification of the larvae in rivers with high diversity is a challenge. Most larvae was identified at family level and it was possible to identify only few genera or species, mostly of the Characidae and Pimelodidae family. The larvae of Siluriformes and Characiformes orders as well as Curimatidae, Auchenipteridae, Pimelodidae, Characidae, Prochilodontidae, Anostomidae and Hemiodontidae families represented together 95% or more of all larvae caught during this study. These results do not corroborate the [4] results, in which the most abundant larvae found in the Solimões River were of the Clupeiformes, Characiformes and Perciformes orders. The small amount of larvae of Siluriformes order must be due to the differences in methodology. Most of the samples in the Solimões River were collected on the surface, while in the present study samples covered all water column. However, the difference

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in the abundance of Clupeiformes and Perciformes larvae in the Solimões River and Madeira River is difficult to be explained only by the methodology and the environmental difference of both rivers must be considered. The selectivity of the method for different species was very clear, while IJ method was very selective for Siluriformes larvae, in particular for the Pimelodidae family; PL method was more selective for the Characiformes larvae, in particular for the Curimatidae and Auchenipteridae families (Table 6). For most families, larvae were found in pre-flexion or earlier stages, except for Characidae and Pimelodidae that showed larvae in more advanced stages, such as *Brycon* spp., *Pimelodus blochii* and *B. rousseauxii*.

The larvae drift indicated the reproductive period of the fish. The IL and PL methods identified a short annual reproductive period for Curimatidae and Prochilodontidae families, between December and April, and a prolonged annual reproductive period for Auchenipteridae and Anostomidae families, between November and May. However, the three methods showed an almost continuous reproductive period for Pimelodidae and Characidae families, with some differences in the moment of the most intensive reproductive peak (Figure 6).

The larval flux estimated by IL and PL methods showed a short annual reproductive period, between December and January, for *Brycon* spp. and *Piaractus brachypomus,* and an annual prolonged reproductive period for *Mylossoma* spp., between December and March. Both methods indicated also a short annual reproductive period for *Triportheus* spp., but IL method indicated March as the reproductive period and PL method indicated May (Figure 8). This difference may be related with the selectivity of the different methods for the three species of *Triportheus* in the area. Thereby, it is necessary to identify the effect of the methods in the evaluation of the larval abundance of those species.

The different methods presented peaks of reproduction activity in different months for *Brachyplatystoma* and *Pimelodus* species (Figure 8), which must be biased by the few number of larvae sampled. The IJ method collected mainly larvae of *P. blochii* and *B. rousseauxii,* and its larval flux showed a biannual short reproductive period for *P. blochii*, in March and in November, and a continuous reproductive period for *B. rousseauxii*, more intensive in the January and decreasing until December. *P. blochii* is considered a species that shows short annual reproductive period, which occurs in the beginning of rainy season [24]. The *P. blochii* larval flux peak occurred in March, at the end of the rainy season, and in November, at the beginning of the rainy season. As the studied area receives the flow discharge of the Beni and Mamoré Rivers, it must be investigated if the two larval peaks found in the area (Figure 8) have originated at the different basins upriver. *B. rousseauxii* reproduces in Andes foothill and the eggs and larvae drift the river toward to Amazon estuary [9]. Studies in Madre de Dios River in Peru, considered the main tributary of the Beni River, showed that this area is a spawning area for *B. rousseauxii* and the reproductive period is prolonged, and spawning period is concentrated at the high water period [5]. The larval flux peak of *B. rousseauxii* larval is in January (Figure 8) and the presence of larvae in advanced developmental stages is in accordance with the drifting movement from the Andes foothill during the high discharge.

Finally, the impact assessment of the new infrastructures projects in the Amazon depends of the data quality obtained before the impact. The present study discussed the effect of the sampling method in the evaluation of the larval drift pattern in the Madeira River. The result

is background knowledge for the future studies to assess the impact of the Jirau hydroelectric power plant to the larval drifting in the Madeira River.

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