Community structure of metazoan parasites from Pimelodus blochii *in two rivers of the Western Brazilian Amazon: same seasonal traits, but different anthropogenic impacts*

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ORIGINAL PAPER

Community structure of metazoan parasites from *Pimelodus blochii* in two rivers of the Western Brazilian Amazon: same seasonal traits, but different anthropogenic impacts

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Abstract

The present investigation evaluated the influence of seasonality and locality on the structure of the parasite community of the catfish *Pimelodus blochii*. A total of 160 fish were collected from two rivers in the State of Acre, western Brazilian Amazon: 80 fish in River Acre and 80 in River Iaco, with 40 in each season (rainy and drought). The overall prevalence was 78.7% and 1461 parasite specimens (adults and larvae) were allocated in 22 taxa: 5 of Monogenea, 10 of Nematoda, 3 of Digenea, 1 of Cestoda and 3 of Crustacea. In drought season, *Procamallanus* (*Spirocamallanus*) *pimelodus* and *Demidospermus peruvianus* were more prevalent in River Acre and Iaco, respectively. The parasite diversity (Brillouin index) as well as the prevalence and abundance of the monogeneans *D. peruvianus*, *D. striatus*, *Demidospermus* sp. and *Ameloblastella* sp. were higher in River Acre. The parasite community structure were dissimilar among rivers, and seasonally in River Iaco. These results suggest that environmental traits may overshadow seasonal influences on the parasite community structure, which may be related to the higher anthropization in River Acre. Furthermore, seasonality exerted less influence on the parasite community than expected, probably because the two rivers have different hydrological traits than those of other rivers in the Brazilian Amazon. New host and locality records expanded our knowledge of parasite biodiversity of *P. blochii*.

Keywords Amazônia Brazil · Freshwater fish · Catfish · Parasite · Ecology

Luciano P. Negreiros and Felipe B. Pereira gave equal intellectual contribution for this publication.

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Introduction

Several factors are known to play some role on the structure of the parasite communities in fish. These factors may be related to the environment (e.g. organic and inorganic conditions) and/or to the host (e.g. feeding, behaviour, physiology, sex) (Blasco-Costa et al. 2014; Oliveira et al. 2017). Furthermore, the abundance of parasites with complex life cycles may be strongly dependent of the population density of intermediate and definitive hosts, superposing time and space (Blasco-Costa et al. 2014).

The Amazon represents one of the greatest flood plain areas associated with large rivers. This region supports the most diverse ichthyofauna in the world, which is composed of species from almost all orders of freshwater fishes (Reis et al. 2001). Through the evolutionary process, the Amazon river basin has been passing through several environmental changes, influencing the ichthyofauna to develop unique physiological, behavioural and biochemical adaptations (Lowe-McConnell 1999). Moreover, the Amazon includes complex environments that, together with the marked flooding oscillations, provide rich resources for fishes (e.g. habitat, shelter and food) influencing their population dynamics directly (Junk 1997).

The parasite community structures of fishes in Amazon have been largely reported oscillating according to seasonal changes, which is annually based on two well-defined periods of rain and drought (Neves et al. 2013; Tavares-Dias et al. 2014; Carvalho and Tavares-Dias 2017). This marked seasonality causes drastic changes in the landscape and is expected to influence all the associated organic and inorganic environmental conditions.

Currently, there are no studies regarding the ecology of parasite communities from the catfish *Pimelodus blochii* Valenciennes (Siluriformes: Pimelodidae); studies have been focused solely on taxonomy of parasites and species catalogue (Gil de Pertierra 2004; Luque and Tavares 2007; Kohn et al. 2007; Luque et al. 2011; Mendoza-Palmero and Scholz 2011; Orélis-Ribeiro and Bullard 2015; Cavalcante et al. 2016, 2018). *Pimelodus blochii* has economic importance and is widely distributed in South America (Lundberg and Littmann 2003; Froese and Pauly 2018); therefore, the community dynamics of parasites from this fish should be better understood. In this sense, the present study evaluated the influences of locality and seasonality of the community structure of the metazoan parasites of *P. blochii* from two different rivers in the State of Acre, western Brazilian Amazon. The main motivation was that the studied areas are under the same seasonal conditions, but show different anthropogenic impacts.

Materials and methods

Study area, sampling of hosts and parasites

Fish were collected with the help of local fisherman, from June 2015 to April 2017, from two different rivers in the State of Acre, Brazil. One collection locality was in the River Acre, Municipality of Rio Branco and the other was in River Iaco, Municipality of Sena Madureira (Fig. 1). According to Pereira and Morais (2015) and Duarte (2017), River Acre is subjected to higher intensity of human activities (i.e. sewer and garbage disposal, agriculture), which results in higher organic and inorganic debris in suspension than in River Iaco. A total of 160 fish were collected: 80 in each river, being 40 during the rainy and 40 during the drought season; the collections were proportionally distributed within each month of the study with respect to the seasonal periods (i.e. 6 to 8 fish collected monthly during 22 months, of which 3 to 4 fish in each locality). Definition of each seasonal period was according to Silva et al. (2008) and Duarte (2017), based on rainfall indices and other climatic variables, in which the rainy season is from November to May and the drought season from June to October.

Fish were kept in small oxygenated water tanks prior to parasitological procedures in the Laboratório de Aquicultura do Instituto Federal de Educação, Ciência e Tecnologia do Acre (acronym IFAC). Hosts were euthanized by spinal cord transection, measured, weighed and analysed for parasites. Gills, skin and digestive tract (oesophagus, stomach, caeca and intestine) were individually observed in Petri dishes containing saline (0.9% NaCl), using a stereomicroscope. Parasites were collected, fixed, stored, quantified and processed for identification according to Eiras et al. (2006).

Voucher specimens were deposited in the Coleção Zoológica de Referência da Universidade Federal do Mato Grosso do Sul (acronym ZUFMS).

Data and statistical analysis

Parasitological terminology used herein follows that of Bush et al. (1997). Species with prevalence < 10% were not included in the statistical analyses. The Shapiro-Wilk test was used to determine whether data of abundance and prevalence of parasites fitted a curve of normal distribution.

A series of preliminary tests were performed to evaluate the homogeneity of host sampling. Differences in the number of individuals from each sex, and length and weight of males and females were tested by locality and seasonality (with overall data and separated by locality), using the Mann-Whitney (U) followed by the post hoc of Dunn when pertinent (Zar 2010). A linear regression was performed to confirm the expected positive correlation between host length and weight. Overall differences in the prevalence and in the mean abundance of parasites from male and female hosts were verified using generalized linear models (GLM), i.e. Poisson regression in the case of abundance and logistic regression in the case of prevalence (see next paragraphs for details). These preliminary tests were performed to avoid biased and/or wrong interpretation of the results, because the analyses were focused on comparing the structure of the parasite community by locality and season.

Differences in prevalence and abundance of parasites were tested between the two sampling areas, and seasonally by each locality, separately. These comparisons were chosen after testing the adequateness of GLMs, based on the Akaike information criteria (Burnham and Anderson 2002) followed by a

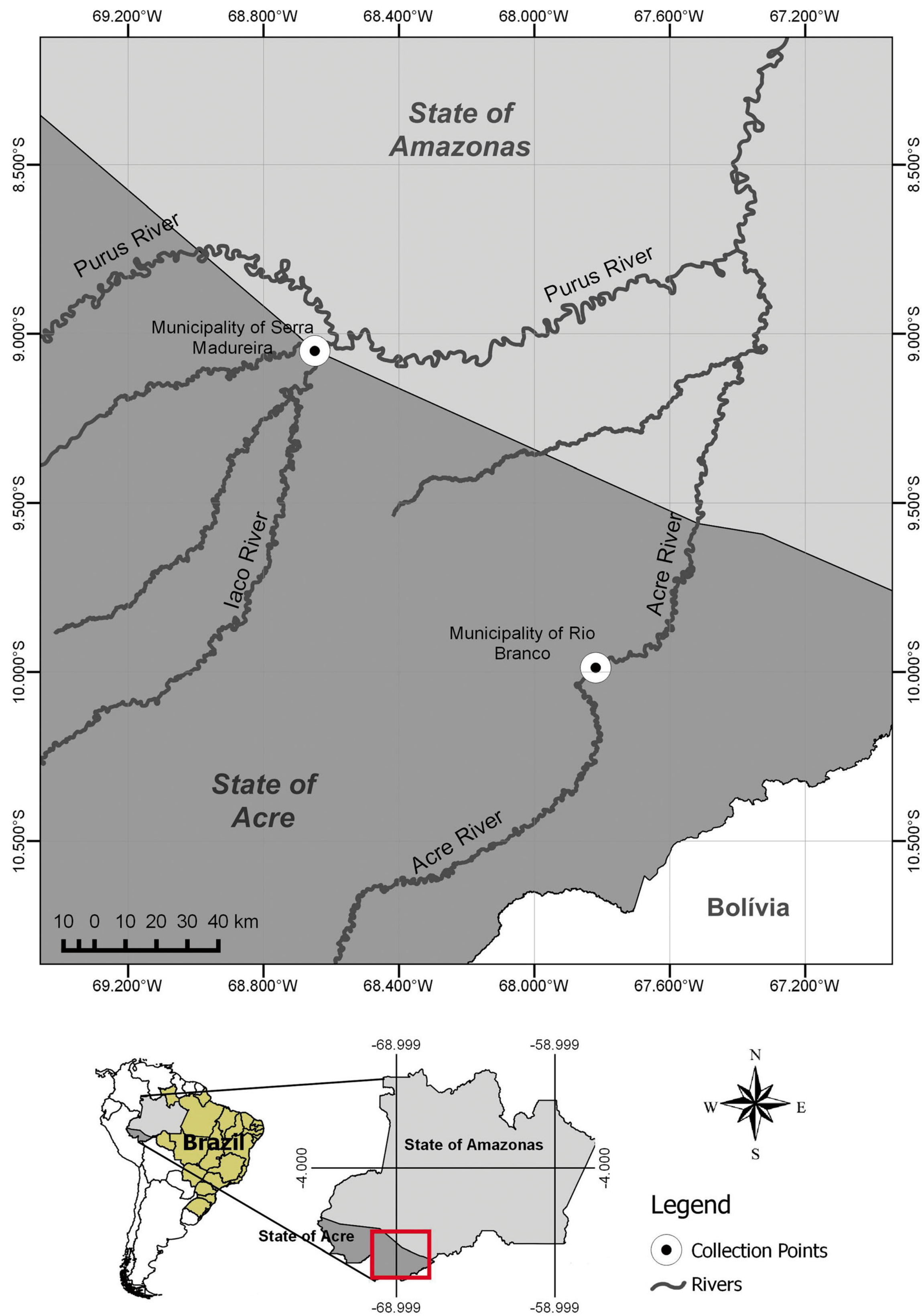

Fig. 1 Map showing the two sampling localities where specimens of *Pimelodus blochii* were collected in the present study

one-way ANOVA, in which the mixed models with interaction of season and locality showed responses statistically similar to the models with season and locality tested separately. These tests were performed using the package lme4 (Bates et al. 2015), implemented in the software R (R Development Core Team 2008). Therefore, the differences in the prevalence were tested using the logistic regression and those in abundance using the Poisson regression (Dohoo et al. 2003). The odds ratio (OR) for each GLM was calculated in order to evaluate the presence and the degree of association between the dependent and the independent variable, where $0 < OR < 1$ indicates negative association, $OR = 1$ indicates lack of association and $OR > 1$ indicates positive association (variables of reference were River Iaco and drought season) (Dohoo et al. 2003). The confidence interval (CI) of 95% for all the GLM was estimated, and if 1 was included in the interval, the model was considered null.

The diversity of parasite infracommunities was estimated using the Brillouin index (HB), and its differences between the two localities and the two seasons within each river separately were evaluated using the *U* test (Zar 2010). The Spearman's rank correlation (rs) was used to verify correlations between host length and parasite diversity (HB), species richness and abundance (overall and of each species) according to the two localities and seasonally by locality, separately (Zar 2010).

Differences in similarity of the parasite component communities were tested using the analysis of similarities (ANOSIM), between the two rivers and seasonally by locality. ANOSIM was based on ranked matrixes, generated from Jaccard index and Bray-Curtis distance (Magurran 2004), with 10,000 permutations.

All the statistical analyses were performed in R (R Development Core Team 2008) and in PAST software (Hammer et al. 2001) with significance level of $p < 0.05$.

Results

Of the 160 *Pimelodus blochii* examined, 117 were females and 43 males. In River Acre, 30 female and 10 male specimens were collected during the rainy season and the same quantity during the drought, totalizing 80 hosts. In River Iaco, 30 females and 10 males were collected in the rainy season and 27 females and 13 males in the drought, totalizing 80 hosts as well. The number of male and female fish were not statistically different between locality and season (Online Resource 1), as well as length and weight were similar between sexes (Online Resources 2, 3); however, fishes were slightly heavier in the rainy period (Online Resource 3). Fish length and weight were positively correlated ($p < 0.001$, $r^2 = 0.86$). Prevalence and abundance of each parasite taxa were not statistically different between male and female hosts (Online Resource 4).

From all of the *P. blochii* examined, 78.7% harboured at least one species of parasite; the overall abundance was 1461, of which 972 specimens parasitizing fish from River Acre (overall prevalence 88.7%) and 489 parasitizing those from River Iaco (68.7% overall prevalence). Twenty-two different taxa were found; the most prevalent species was the nematode *Procamallanus* (*Spirocamallanus*) *pimelodus* Pinto, Fábio, Noronha & Rolas, 1974 (48.8%) followed by the monogenean *Demidospermus peruvianus* Mendoza-Palmero & Scholz, 2011 (32.5%); 4 taxa were found as larvae, three nematodes and one digenetic (Table 1). Seventeen taxa co-occurred in both component communities from River Acre and River Iaco (Table 1).

Differences in prevalence and abundance of parasites were more evident comparing the two localities rather than seasonally, in which monogeneans showed higher prevalence and abundances in River Acre (Table 1). Locally, during the drought season, *P.* (*S.*) *pimelodus* and *D. peruvianus* were more prevalent in River Acre and River Iaco, respectively (Table 2).

The mean diversity was higher in River Acre than in River Iaco ($H = 0.7 \pm 0.6$ vs. 0.4 ± 0.4, respectively; $U = 2035.5$, $p < 0.001$), as well as during the drought in River Iaco ($H = 0.49 \pm 0.48$ vs. 0.24 ± 0.36 in drought and rainy season respectively; $U = 565$, $p = 0.014$), but was not different among seasons in the River Acre ($H = 0.75 \pm 0.63$ vs. 0.74 ± 0.54 in rainy and drought season respectively; $U = 776$, $p = 0.83$). There was no correlation between host length and parasite diversity (H), species richness and abundance in any of the comparisons performed (see Online Resource 5 for all the related results).

The ANOSIM results indicated no qualitative and quantitative similarities comparing the component communities from the two rivers (Jaccard index matrix $R = 0.075$, $p = 0.0001$; Bray-Curtis distance matrix $R = 0.090$, $p = 0.001$), as well as comparing the component communities between seasons in the River Iaco (Jaccard index matrix $R = 0.026$, $p = 0.045$; Bray-Curtis distance matrix $R = 0.027$, $p = 0.045$). However, the ANOSIM indicated similarity among rainy and drought periods in River Acre (Jaccard index matrix $R = 0.008$, $p = 0.063$; Bray-Curtis distance matrix $R = 0.009$, $p = 0.089$).

Discussion

Almost 60% of the parasite species found in the present study have been reported in *P. blochii* in different regions of South America (Gil de Pertierra 2004; Mendoza-Palmero and Scholz 2011; Orélis-Ribeiro and Bullard 2015). However, the monogeneans *D. peruvianus*, *D. striatus* Mendoza-Palmero and Scholz, 2011, and *Scleroductus* sp., the nematode *Raphidascaris* sp. and the crustaceans *Dipteropeltis* sp., *Ergasilus* sp. and *Minilernea floricapitella* Thatcher & Huergo, 2005, represent new host records for *P. blochii* in Brazil. Furthermore, the nematodes

Table 1 Prevalence (P), mean abundance (MA) ± 1 standard deviation (SD), values of odds ratio (OR), confidence interval (CI) with 95% of confidence and *p* of the respective logistic and Poisson regression associated to the metazoan parasites of *Pimelodus blochii* (*n* = 160), from River Acre (*n* = 80) and River Iaco (*n* = 80), State of Acre, western Brazilian Amazon. Species with overall prevalence < 10% were not included in the regression analyses; values in italic are statistically significant

		River Acre		River Iaco		Logistic regression			Poisson regression		
Parasite species	Site of infection	P (%)	MA ± SD	P (%)	MA ± SD	OR	CI	*p*	OR	CI	*p*
Monogenea											
Demidospermus peruvianus	Gills	45.0	1.9 ± 4.0	21.2	0.4 ± 1.2	0.30	0.22–0.31	*< 0.001*	0.80	0.73–0.92	*0.018*
Demidospermus striatus	Gills	20.0	1.0 ± 4.3	6.2	0.1 ± 0.4	0.30	0.20–0.33	*< 0.001*	0.80	0.74–0.90	*0.018*
Demidospermus sp.	Gills	38.7	0.9 ± 1.5	5.0	0.08 ± 0.4	0.08	0.03–0.10	*< 0.001*	0.46	0.15–0.50	*0.006*
Ameloblastella sp.	Gills	32.5	1.1 ± 3.8	3.7	0.05 ± 0.3	0.08	0.05–0.22	*< 0.001*	0.33	0.22–0.51	*0.013*
Scleroductus sp.	Gills	1.2	0.01 ± 0.1	1.2	0.01 ± 0.1	–	–	–	–	–	–
Nematoda											
Procamallanus (*S.*) *rarus*	Small intestine	7.5	0.2 ± 0.9	8.7	0.2 ± 0.6	–	–	–	–	–	–
Procamallanus (*S.*) *pimelodus*	Small intestine	55.0	2.5 ± 3.9	42.5	2.4 ± 6.3	0.60	0.50–1.1	0.11	0.98	0.87–1.3	0.58
Cucullanus pinnai	Large intestine	10.0	0.2 ± 0.6	11.2	0.1 ± 0.4	1.14	0.99–1.3	0.79	0.89	0.73–2.3	0.06
Orientatractis moraveci	Large intestine	6.2	0.1 ± 0.4	1.2	1.0 ± 0.1	–	–	–	–	–	–
Philometroides acreanensis	Stomach wall	–	–	6.2	0.1 ± 0.4	–	–	–	–	–	–
Rondonia rondoni	Intestine	7.5	0.07 ± 0.7	5.0	1.1 ± 9.5	–	–	–	–	–	–
Anisakidae gen. sp. (larva)	Mesentery	20.0	0.8 ± 3.6	7.5	0.2 ± 0.6	0.45	0.22–1.4	0.14	0.85	0.80–1.93	0.34
Pharyngodonidae gen. sp.	Large intestine	16.2	1.5 ± 6.0	5.0	0.05 ± 0.2	0.27	0.23–1.2	0.06	0.60	0.55–1.43	0.14
Spiruroidea gen. sp. (larva)	Large intestine	2.5	0.03 ± 0.2	–	–	–	–	–	–	–	–
Raphidascaris sp.(larva	Large intestine	–	–	1.2	1.0 ± 0.1	–	–	–	–	–	–
Digenea											
Prosthenhystera obesa	Gallbladder	2.5	0.02 ± 0.2	–	–	–	–	–	–	–	–
Dadaytrema sp.	Large intestine	20.0	0.9 ± 5.7	10.0	1.0 ± 4.4	0.44	0.31–1.47	0.08	1	0.90–1.44	0.96
Metacercariae type-*Diplostomulum*	Gills, liver	6.2	0.08 ± 0.4	1.2	0.01 ± 0.1	–	–	–	–	–	–
Cestoda											
Monticellia magna	Small intestine	3.7	0.06 ± 0.4	15.0	0.2 ± 0.6	–	–	–	–	–	–
Crustacea											
Dipteropeltis sp.	Gills	1.2	0.01 ± 0.1	–	–	–	–	–	–	–	–
Ergasilus sp.	Gills	8.7	0.1 ± 0.4	3.7	1.0 ± 0.1	–	–	–	–	–	–
Minilernaea floricapitella	Gills	–	–	1.2	1.0 ± 0.1	–	–	–	–	–	–

Cucullanus pinnai pinnai Travassos, Artigas & Pereira, 1928, *P.* (*S.*) *pimelodus* and *P.* (*S.*) *rarus* Travassos, Artigas & Pereira, 1928, and the cestode *Monticellia magna* (Rego, dos Santos & Silva, 1974) infecting *P. blochii* in the State of Acre expand the geographic range of these parasites to the western Amazon.

The parasite community of *P. blochii* showed a richer fauna of endoparasites, but most of the species of monogeneans were highly prevalent (see Table 1). Moreover, almost 18% of all parasite specimens were found as larval stages. These results indicate that *P. blochii* has important and different roles on the transmission of the present parasite species, acting as intermediate, paratenic and/or definitive host, and the transmission patterns are largely linked to the omnivory of the fish (Sánchez-Botero and Araújo-Lima 2001). In this sense, infections by endoparasites that exhibit diverse life cycles and different transmission mechanisms may be favoured (Moravec 1998; Tavares-Dias et al. 2014; Oliveira et al. 2017), superposing the richness of ectoparasites.

The Amazon has a well-defined seasonality, which is based on periods of intense rainfall followed by those of drought, and these characteristics are known to commonly influence the structure of parasite communities in the biome (Neves et al. 2013; Tavares-Dias et al. 2014; Carvalho and Tavares-Dias 2017). However, the present results showed some different and interesting patterns rather than the expected regarding the River Acre, where variations in the parasite community were very slight.

In the River Acre, the only seasonal variation observed was in the prevalence of *P.* (*S.*) *pimelodus* that was higher during the drought season. This result may be related to the host dietary habits, because fish are predominantly herbivorous in the rainy season, but generalists in the drought (López-Casas and Jiménez-Segura 2007; Soares et al. 2011) which increases the probability of ingesting intermediate hosts harbouring *P.* (*S.*) *pimelodus* infective larvae.

In River Iaco, parasite communities were seasonally dissimilar, with higher prevalence of *D. peruvianus* in the drought resulting in higher diversity in this season. During the drought period, flooded areas are reduced, consequently favouring the fish aggregation and the transmission of ectoparasites with direct life cycle (e.g. monogeneans) as has been observed in the Brazilian Amazon (Neves et al. 2013; Amarante et al. 2015).

Several factors determine differences in the structure of parasite communities of hosts from different populations and localities (Oliveira et al. 2017; Yamada et al. 2017). The

Table 2 Prevalence (P), mean abundance (MA) ± 1 standard deviation (SD), values of odds ratio (OR), confidence interval (CI) with 95% of confidence and *p* of the respective logistic and Poisson regression associated to the metazoan parasites of *Pimelodus blochii* (n = 160), from River Acre (*n* = 80) and River Iaco (*n* = 80), State of Acre, western Brazilian Amazon, compared according to rainy (*n* = 40 from each locality) and drought (*n* = 40 from each locality) seasons. Species with prevalence < 10% according to each locality were not included in the regression analyses and are not shown; values in italic are statistically significant. *M*, Monogenea; *N*, Nematoda; *D*, Digenea

	Rainy season		Drought season		Logistic regression			Poisson regression		
Parasite species	P (%)	MA ± SD	P (%)	MA ± SD	OR	CI	*p*	OR	CI	*p*
River Acre										
Demidospermus peruvianus (M)	33.0	2.4 ± 3.5	30.0	3.1 ± 7.7	1.5	0.62–3.63	0.37	0.99	0.93–1.04	0.72
Demidospermus striatus (M)	18.0	0.8 ± 2.7	23.0	1.2 ± 5.5	1.2	0.61–3.44	0.33	0.99	0.93–1.01	0.73
Demidospermus sp. (M)	43.2	1.4 ± 2.2	35.4	0.9 ± 1.9	1.4	0.56–3.38	0.49	1.04	0.91–1.19	0.51
Ameloblastella sp. (M)	33.1	0.7 ± 1.2	30.0	0.8 ± 2.3	1.1	0.44–2.89	0.80	0.97	0.79–1.17	076
Procamallanus (*S.*) *pimelodus* (N)	43.5	2.1 ± 4.0	68.7	4.4 ± 7.5	3.6	2.14–3.88	*0.02*	0.99	0.95–1.02	0.23
Anisakidae gen. sp. (N)	23.0	0.5 ± 1.3	8.9	0.2 ± 1.0	3.5	0.9–14.39	0.07	1.10	0.89–1.37	0.37
Pharyngodonidae gen. sp. (N)	23.6	2.0 ± 5.8	10.0	1.1 ± 6.2	2.6	0.73–9.32	0.13	1.10	0.97–1.06	0.63
Dadaytrema sp. (D)	18.3	1.7 ± 8.1	23.0	0.3 ± 0.7	0.7	0.24–2.2	0.57	1.01	0.97–1.05	0.57
River Iaco										
Demidospermus peruvianus (M)	8.0	0.1 ± 0.4	33.0	0.7 ± 2.2	2.2	1.92–2.65	*< 0.001*	0.55	0.23–1.27	0.15
Cucullanus pinnai (N)	8.5	0.1 ± 0.3	15.0	0.2 ± 0.5	0.5	0.12–1.98	0.29	0.6	0.21–1.86	0.39
Procamallanus (*S.*) *pimelodus* (N)	40.0	2.2 ± 5.3	38.0	2.7 ± 7.2	1.5	0.62–3.68	0.37	0.5	0.23–1.27	0.37

present results showed a more diverse parasite fauna in River Acre, as same as the ANOSIM indicated no similarity among the two rivers. Even though *P. blochii* migrates during the breeding season and may overlap the two sampling areas (López-Casas and Jiménez-Segura 2007; Soares et al. 2011), the local conditions seem to be important on structuring the studied component community. An example is that monogeneans were more prevalent and abundant in River Acre as they are monoxenic ectoparasites in unremitting contact with the external environment. Moreover, the nearly complete lack of seasonal variations in the parasite community structure in River Acre compared with those in River Iaco may be related to the different anthropogenic impacts that are higher in River Acre, drastically increasing the amount of organic and inorganic sediments in suspension (Pereira and Morais 2015; Duarte 2017). Therefore, even though both rivers are in the same basin and exhibit similar limnology and hydrology (Silva et al. 2008), the impact of human activities may cause significant environmental changes on a local scale (close to cities and/or areas of agriculture). These issues support the idea that the local environment has influence on the parasite component community of the present study and, in River Acre, it seems to be stronger than the seasonal variations.

It should be mentioned that fishes were heavier in the rainy period, probably because it represents the breeding season and flooded areas for foraging are increased (López-Casas and Jiménez-Segura 2007; Soares et al. 2011; Froese and Pauly 2018). However, as the fish weight showed strong and positive correlation with fish length, which had no influence on the parasite fauna (see Online Resource 3), it is clear that fish weight is not determinant in the structure of this parasite community.

Based on the present results, the seasonal influence on the component community structure of parasites was less intense than expected, contrasting with similar approaches in the Amazon (Neves et al. 2013; Tavares-Dias et al. 2014; Carvalho and Tavares-Dias 2017). Therefore, it should be highlighted that River Acre and Iaco, both tributaries of River Purus, have their headwaters in Peru, at the base of the Andes mountain range, resulting in different hydrological and fluvial level fluctuations when compared with those from most of the other rivers in the Brazilian Amazon (Bardales et al. 2010; Wadt et al. 2010). Most likely, this resulted in the less intense seasonal influence observed here, compared with studies in other Amazonian sub basins (see Neves et al. 2013; Tavares-Dias et al. 2014; Carvalho and Tavares-Dias 2017).

These results were generated from a 2-year sampling, and despite that sampling data was robust, in a longer temporal scale, data may exhibit different behaviour as the low *R* values of ANOSIM suggested that other factors than locality and seasonality are influencing the homogeneity of the parasite communities. Nevertheless, the higher values of prevalence and abundance of monogeneans in River Acre (more anthropized) contrasting with those in Rive Iaco (less anthropized) suggest a possible biological indicator role for these parasites as asserted by other authors (Sanchez-Ramires et al. 2007; Palm 2011). Finally, these results expanded our knowledge of parasite biodiversity in *P. blochii*, an economically important species with farming potential.

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Compliance with ethical standards

Ethical Disclosures All procedures involving animals were authorised by the Instituto Chico Mendes para Conservação da Biodiversidade (acronym SISBIO, N° 60899-1) and were strictly according to the protocols and rules of the Committee on Ethics of Animal Use of the Embrapa Amapá (Protocol No. 002-CEUA-CPAFAP).

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