

# Diversity of parasites in *Cichlasoma amazonarum* Kullander, 1983 during rainy and dry seasons in eastern Amazon (Brazil)

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## Summary

This study investigated the influence of the rainy and dry seasons on the parasite communities of *Cichlasoma amazonarum* (Kullander, 1983) in a tributary of the Amazon River system, northern Brazil. Of 112 fish examined, 95.5% were parasitized by *Ichthyophthirius multifiliis*, *Piscinoodinium pillulare*, *Gussevia disparoides*, *Posthodiplostomum* sp., *Procamallanus (Spirocamallanus) inopinatus*, and *Echinorhynchus paranensis*, as well as by the Glossiphoniidae leech. *Ichthyophthirius multifiliis* and *P. pillulare* were the dominant parasite species, with *I. multifiliis* the most prevalent and abundant; the leech (Glossiphoniidae) was the least prevalent and least abundant. Parasites presented an aggregate dispersion pattern and seasonal variations in infestation levels, influenced by the environmental conditions in the rainy season. Variation in the parasite dynamics created overall changes in the parasite communities, characterized by greater diversity, species richness and evenness during the rainy season. However, there was a high similarity (99.8%) in the parasite community structure between the rainy and dry seasons. During the dry season the hosts had predominantly 1–3 parasite species compared to 3–4 parasites in the rainy season. Some parasites in the eastern Amazon undergo population changes relating to seasonality. These results thus indicate the correct season to apply adequate prophylactic measures to reduce negative impacts of parasites in this wild ornamental fish when captured.

## 1 | INTRODUCTION

Studies conducted in the eastern Amazon (Brazil) have indicated that the parasite communities of native cichlids do not present a diversity of species, but rather consist of a small number of specialist species (Neves, Pereira, Tavares-Dias, & Luque, 2013; Tavares-Dias, Oliveira, Gonçalves, & Silva, 2014). However, there have been few studies on the seasonal variations of the parasite community in native cichlids in the Amazon region (Neves et al., 2013; Tavares-Dias et al., 2014), even though this Neotropical region contains more than 180 species of these Cichliformes, some of which are endemic (Froese & Pauly, 2016). Thus, the seasonal variation of the parasite prevalence and their abundance in *Cichlasoma amazonarum* Kullander, 1983 should be investigated.

Temperature can influence the immune system of the host fish and the reproduction rate of the parasites, given that innate immunity is

more active at low temperatures while immunity of the adaptive system tends to be suppressed at low temperatures (Dias & Tavares-Dias, 2015; Ondracková et al., 2015; Rohlenová et al., 2011). Therefore, in temperate climate regions, the seasonal temperature variations may alter not only the metabolism of the fish, but also the parasite communities in fish populations (Lamková, Šimková, Palíková, Jurajda, & Lojek, 2007; Ondracková et al., 2015; Rohlenová et al., 2011). However, in regions with a tropical climate, it can be expected that the parasite communities will have a stable structure over the course of the year (Dias & Tavares-Dias, 2015; Violante-González, Aguirre-Macedo, & Rojas-Herrera, 2008). In the Amazon region, since the climate is one of tropical forest, the dynamics of rainfall levels may promote changes to the quality of the environment in the different ecosystems (Dias & Tavares-Dias, 2015; Gonçalves, Oliveira, Neves, & Tavares-Dias, 2016; Neves et al., 2013; Tavares-Dias et al., 2014). The rainy season lasts from December to May and the dry season from June to November

(Souza & Cunha, 2010), thus promoting changes to the Amazonian fish community and affecting some parasites (Dias & Tavares-Dias, 2015; Gonçalves et al., 2016; Neves et al., 2013; Tavares-Dias et al., 2014).

Due to fishery of *C. amazonarum* and with gradual increase in the aquaculture of this fish, there is a need to determine the seasonal variation of their parasites. This is critical to assess the parasites' reproductive cycles and design adequate mitigation methods. We hypothesized that some parasite species vary during the rainy or dry season. Therefore, the parasite community and effects of seasons on the variability of the parasites in wild *C. amazonarum* were studied during the rainy and dry seasons in a tributary of the Amazon River system in eastern Amazon, northern Brazil.

## 2 | MATERIALS AND METHODS

### 2.1 | Study area

This study was conducted at three points in the Igarapé Fortaleza basin (00°00'56.3N; 051°05'27.1W), a system formed by a main channel and rich swamps influenced by rainfall in the Amazon region and the tides of the Amazon River. Fish were caught at night using gill nets of different mesh sizes (15–35 mm). Regional vegetation consists of plants characteristic of floodplain forests and herbaceous fields that are periodically inundated, bringing in many species of macrophytes (Tavares-Dias et al., 2014; Thomaz, Costa-Neto, & Tostes, 2004). The climate is typical for this tropical forest, with maximum rainfall rates from December to May and minimums from June to November (Souza & Cunha, 2010). Seasonality was therefore based on the rainy and dry seasons. This seasonality promotes a transition within the flooded system of the vegetation, land and water, thereby creating a very dynamic ecosystem. At the time of fish capture, the pH, temperature and dissolved oxygen of the water were measured using digital devices appropriate for each purpose. The monthly rainfall rates were obtained from the Hydrometeorology and Renewable Energy Center (NHMET) of the Institute of Scientific and Technological Research of the State of Amapá (IEPA). From July 2011 to February 2013, the water quality data were measured once each month.

### 2.2 | Fish and parasite sampling procedures

From July 2011 to February 2013, 112 specimens of *C. amazonarum* were captured for parasitological analyses. Fish collected in the dry season measured  $12.5 \pm 2.1$  cm (range 8.2–18.9) total length and weighed  $47.1 \pm 19$  g (range 7.6–79.4); fish collected in the rainy season measured  $12.6 \pm 1.3$  cm (range 9.0–14.5) total length and weighed  $54.3 \pm 16.5$  g (range 24.0–108). All fish were conserved for 2–3 hr in ice and transported to the Laboratory for Aquatic Organism Health, at Embrapa Amapá. The mouth, opercula and gills of each necropsied fish were examined to detect the presence of ectoparasites, and the viscera and gastrointestinal tract were examined to detect endoparasites. Collection, fixation, counting, and preparation of parasites for identification were according to techniques described by Eiras, Takemoto, and Pavanelli (2006). Protozoan parasites were

counted in a Sedgewick-Rafter chamber. The parasitological terms used are in accordance with Rohde, Hayward, and Heap (1995) and Bush, Lafferty, Lotz, and Shostak (1997).

### 2.3 | Data analysis

Descriptors for the constituent parasite community were calculated: (i) species richness; (ii) Brillouin diversity index (*HB*); (iii) evenness associated with the Brillouin diversity index (*E*); and (iv) Berger-Parker dominance index (*d*) (Magurran, 2004), using the Diversity software (Pisces Conservation Ltd, UK). Similarity in parasite community structure between the rainy and dry season was compared using two different distance indices: the Jaccard index (qualitative index), based on presence-absence data; and the quantitative Bray-Curtis index, based on parasites abundance (Ludwig & Reynolds, 1988; Magurran, 2004). These similarity indices were determined using Past software (Paleontological Statistics version 3.0).

Differences in parasite prevalence between the rainy and dry seasons were evaluated using the chi-square test ( $\chi^2$ ) with Yates correction, and differences in abundance using the Mann-Whitney test (*U*). The Shapiro-Wilk test was used to determine whether the parasite abundance presented normal distribution. Spearman coefficient (*rs*) was used to investigate the correlation of host length to abundance, species richness, *HB* and *E*. Total length and weight of the host between dry and rainy seasons were compared using the *t*-test. Body weight (g) and total length (cm) were used to calculate the relative condition factor (*Kn*) of the fish using the length-weight relationship ( $W = aL^b$ ) after logarithmic transformation of length and weight and subsequent adjustment of two straight lines, thus obtaining  $\ln y = \ln a + \ln x$  (Le-Cren, 1951). The relative condition factor values for the fish in the dry and rainy seasons were compared using the Mann-Whitney test (*U*) (Zar, 2010).

## 3 | RESULTS

During the rainy season, the mean water temperature was  $29.5 \pm 0.3^\circ\text{C}$ ; pH  $6.1 \pm 0.4$ ; dissolved oxygen  $2.6 \pm 0.3$  mg/L and rainfall  $232.9 \pm 158.9$  mm. During the dry season, the mean water temperature was  $30.5 \pm 0.3^\circ\text{C}$ ; pH  $6.3 \pm 0.3$ ; dissolved oxygen  $2.0 \pm 0.3$  mg/L and rainfall  $88.6 \pm 100.6$  mm.

Among the 112 *C. amazonarum* specimens necropsied, all were parasitized by one or more species. A total of 1,273,499 parasites was collected, including *Ichthyophthirius multifiliis*, *Piscinoodinium pillulare*, *Gussevicia disparoides*, *Posthodiplostomum* sp., *Procamallanus* (*Spirocamallanus*) *inopinatus*, *Echinorhynchus paranensis* and the leeches Glossiphoniidae gen. sp. The dominant species was *I. multifiliis*, and the constituent community was dominated by ectoparasite species.

Parasitism by *P. (S.) inopinatus* and *E. paranensis* showed no seasonal variation, with a low prevalence and abundance during both dry and rainy seasons. In contrast, the highest prevalence and abundance of *G. disparoides* and metacercariae of *Posthodiplostomum* sp. were during the rainy season. The highest prevalence of *P. pillulare* was

**TABLE 1** Differences in prevalence (P) and mean abundance (MA) ± SD between seasons for parasites of *Cichlasoma amazonarum*, eastern Amazon, northern Brazil, July 2011 to February 2013

Species of parasites	IS	Dry season (N = 61)		Rainy season (N = 51)		$\chi^2$	U
		P (%)	MA	P (%)	MA		
<i>Ichthyophthirius multifiliis</i>	Gills	96.7	20,155.4 ± 49,785.1	92.1	364.3 ± 400.2	1.1	2850.0**
<i>Piscinoodinium pillulare</i>	Gills	8.2	116.2 ± 550.2	49.0	24.0 ± 37.6	23.6**	980.0**
<i>Gussevia disparoides</i>	Gills	23.0	1.98 ± 8.7	82.4	25.6 ± 37.5	39.2**	489.5**
Glossiphoniidae gen. sp.	Gills	0	0	2.0	0.0 ± 0.1	1.2	1525.0
<i>Posthodiplostomum</i> sp. (metacercariae)	Gills	47.5	36.6 ± 179.3	92.2	244.8 ± 541.8	25.3**	690.0**
<i>Posthodiplostomum</i> sp. (metacercariae)	Intestine	4.9	0.05 ± 0.2	25.5	18.5 ± 84.3	10.9**	1188.5**
<i>Procamallanus</i> (S.) <i>inopinatus</i> (larvae)	Intestine	9.8	0.2 ± 0.7	21.6	0.3 ± 0.7	2.9	1375.5
<i>Echinorhynchus paranensis</i> (larvae)	Intestine	1.6	0.1 ± 0.9	7.8	0.1 ± 0.5	2.5	1860.5

IS = infection site, U = Mann-Whitney test,  $\chi^2$  = chi-square test. \*\* $p < .001$ .

**TABLE 2** Differences in diversity indices between seasons for parasites collected from *Cichlasoma amazonarum*, eastern Amazon, northern Brazil, July 2011 to February 2013

Diversity indices	Dry season (N = 61)	Rainy season (N = 51)	U	p
Species richness	1.9 ± 1.0 (1–5)	3.7 ± 1.0 (2–7)	353.0	.001
Brillouin (HB)	0.09 ± 0.22 (0.003–0.89)	0.49 ± 0.31 (0.02–1.20)	288.0	.001
Evenness (E)	0.05 ± 0.11 (0–0.43)	0.25 ± 0.15 (0.01–0.63)	287.0	.001
Berger-Parker (d)	0.96 ± 0.11 (0.52–1.00)	0.82 ± 0.15 (0.46–1.00)	2783.0	.001

U = Mann-Whitney test; p = probability. Values in parentheses indicate range.

also during the rainy season, but with the highest intensity during the dry season; the highest prevalence of *I. multifiliis* was during the dry season. Glossiphoniidae species were leeches found at low levels of parasitism on the gills of *C. amazonarum*, and only in the rainy season (Table 1).

There was a variation in the parasitic diversity indices between the rainy and dry seasons, whereby the diversity index (HB), evenness of diversity (E) and parasite species richness had higher values during the rainy season. In contrast, the Berger-Parker dominance (d) was higher during the dry season (Table 2).

Host length showed no correlation with parasite species richness ( $r_s = .028$ ,  $p = .771$ ), Brillouin diversity ( $r_s = .060$ ,  $p = .529$ ) or evenness ( $r_s = .043$ ,  $p = .653$ ). During the dry season, the hosts were predominantly infected by 1–3 parasite species; during the rainy season, the hosts had predominantly 3–4 parasite species (Figure 1). The Jaccard index ( $J = 1.0$ ) and Bray-Curtis dissimilarity ( $B = 0.17$ ) showed a high similarity in the abundance of the parasite community between the rainy and dry seasons.

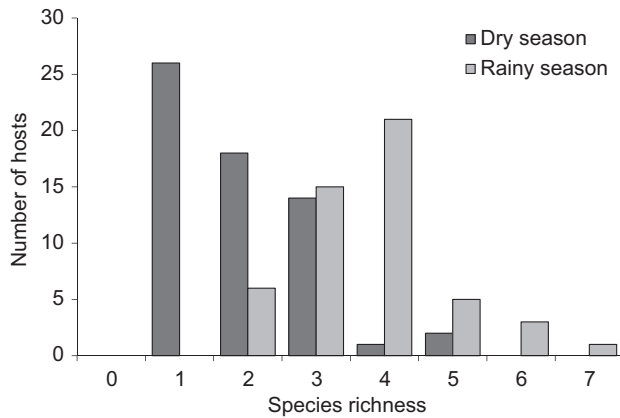
For the hosts, growth was allometrically negative ( $b = 1.6921$ ;  $r^2 = .813$ ), and body weight was positive in relation to total length ( $r_s = .872$ ,  $p < .001$ ). This negative allometric growth indicated that there was a greater increase in body weight than in size. However, the fish condition factor in the dry season was higher than in the rainy

season, thus indicating different body conditions (Figure 2). In contrast, the weight of the host in the dry season was lower ( $t = 2.09$ ,  $p = .039$ ) than in the rainy season.

Abundance of *G. disparoides* presented a weak correlation with the length and weight of the host, as did the abundance of *Posthodiplostomum* sp. in the gills in relation to the length and weight of the host. In contrast, the abundance of *Posthodiplostomum* sp. in the intestine presented no correlation with length and weight of the host. The abundance of *I. multifiliis*, *P. pillulare*, and *P. (S.) inopinatus* showed no correlation with the length and weight of the host (Table 3).

## 4 | DISCUSSION

The parasite community of *C. amazonarum* consists mostly of ectoparasite species. In addition, the length of the host did not influence the richness of parasite species, Brillouin diversity index, or evenness, with the highest values occurring during the rainy season. The parasite community in cichlid *Aequidens tetramerus* in the same region as that of the present study was composed predominately of ectoparasites species, presenting greater richness of species, diversity and evenness during the dry season (Tavares-Dias et al., 2014). In contrast, *Cichlasoma trimaculatum* in a lake in Mexico had a parasite community composed



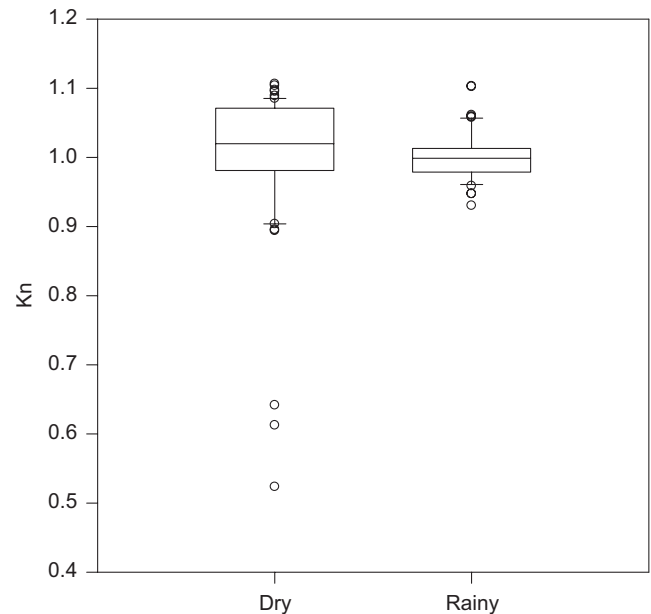
**FIGURE 1** Species richness of *Cichlasoma amazonarum* parasites during dry and rainy seasons, eastern Amazon, northern Brazil, July 2011 to February 2013

**TABLE 3** Spearman correlation coefficient ( $r_s$ ) of *Cichlasoma amazonarum* parasite abundance as to length and weight, eastern Amazon, northern Brazil, July 2011 to February 2013

Body parameters	Total length		Body weight	
	$r_s$	$p$	$r_s$	$p$
Parasite species				
<i>Ichthyophthirius multifiliis</i>	.166	.081	.169	.169
<i>Piscinoodinium pillulare</i>	.045	.639	-.159	.094
<i>Gusseviva disparoides</i>	-.211	.025	-.217	.021
<i>Posthodiplostomum</i> sp.	-.226	.016	-.324	.0005
<i>Procammallanus</i> (S.) <i>inopinatus</i>	-.050	.596	.009	.923

predominately of endoparasite species, with greater parasite richness and diversity during the rainy season (Violante-González et al., 2008). The parasitic communities are influenced by various biotic and abiotic factors (Dias & Tavares-Dias, 2015; Ondracková et al., 2015; Rohlenová et al., 2011); hence, they can cause differences among different fish populations. Seasonal variations in parasite communities have been usually correlated with environmental changes. In a given parasite community, some species undergo substantial changes relating to seasonality, while others do not. This has been explained primarily by parasite life cycles, environmental dynamics and host-specific immune responses (Lamková et al., 2007; Neves et al., 2013; Ondracková et al., 2015; Tavares-Dias et al., 2014; Violante-González et al., 2008).

The *C. amazonarum* parasite community presented only one specialist parasite: the *G. disparoides*; the component community presented the same pattern in this cichlid, i.e. with a highly dominant species and little diversity. *Cichlasoma amazonarum* feeds on aquatic plants, seeds, insects and detritus (Soares et al., 2011), but its omnivorous diet also includes small invertebrates (microcrustaceans and mollusks), as indicated in this study. The endoparasite communities, which are trophically transmitted parasites, indicate that this host consumed a low diversity of prey in the studied habitat, which suffers from an urban anthropogenic eutrophication that is greater during the dry season (Gonçalves et al., 2016; Takyama, Silva, Costa, & Nascimento,



**FIGURE 2** Relative condition factor ( $K_n$ ) for *Cichlasoma amazonarum*, eastern Amazon, northern Brazil, July 2011 to February 2013. Box plots represent medians, interquartile ranges, minimum–maximum ranges and outliers. Different mean values between dry ( $n = 61$ ) and rainy ( $n = 51$ ) season according to the Mann–Whitney test ( $U = 1886.0$ ,  $p = .033$ )

2004; Tavares-Dias et al., 2014). This may have an effect on the parasite community.

In the Amazon region, seasonality is strongly influenced by the rainy and dry seasons. In *C. amazonarum* there was a high similarity (almost 100%) in the parasite community structure between the rainy and dry seasons; one of the factors influencing the parasites might be the level of rainfall. The prevalence of *P. pillulare* in *C. amazonarum* was highest in the rainy season, while abundance was higher in the dry season. In addition, *I. multifiliis*, the most prevalent and dominant parasite, occurred during both the rainy and dry seasons, but its abundance was greater during the rainy season. The high abundance of *I. multifiliis* during the dry season is due to low environmental quality, which seems to affect different hosts differently over the course of time. In addition, there were higher levels of parasitism due to *G. disparoides* in *C. amazonarum* during the rainy season, when the environmental quality was better. Similar studies focusing on directly transmitted parasites have also reported a relationship between prevalence and abundance of ectoparasites in the dry/rainy season cycle in the Amazon (Gonçalves et al., 2016; Neves et al., 2013; Tavares-Dias et al., 2014). However, other factors may also affect the prevalence and abundance of parasites in host fish populations. Thus, further studies are necessary, as we need to have a more comprehensive sampling strategy and with a larger sample size to be able to know how these parameters affect the parasite load in terms of reproduction, sex and age of *C. amazonarum* and in interaction with others factors, e.g. the temporal variation.

The dynamics of the process of recruitment of *Posthodiplostomum* sp. in mollusks and maintenance of metacercariae populations in the intermediate and definitive hosts possibly vary with the Amazonian

seasonal cycle (Gonçalves et al., 2016; Neves et al., 2013). However, in *C. amazonarum*, there were higher parasitic levels of metacercariae of *Posthodiplostomum* during the rainy season. In addition, the highest parasitic levels of this digenean were found mainly in smaller fish. Therefore, because these infestations come into direct contact with cercariae of *Posthodiplostomum* sp., the hydrological cycle, hosts' feeding pattern and seasonal availability of the infectious stages of parasites in the environment were determining factors for the seasonal variations of these endoparasites. Environmental conditions may also play an important role in determining seasonal variation in prevalence and abundance of digeneans (Gonçalves et al., 2016; Violante-González et al., 2008). Moreover, this first report of *E. paranensis* in *C. amazonarum* showed low parasitic levels of this acanthocephalan, and thus had no effect in the dry and rainy seasons. These low levels of parasitism caused by *E. paranensis* larvae and adults in both seasons indicate that *C. amazonarum* is a paratenic host for this acanthocephalan, with an unknown life cycle.

In *C. amazonarum*, parasitic levels of *P. (S) inopinatus* were low, occurring in both the rainy and dry seasons. Such results therefore indicate the frequent presence of chironomids infected with *P. (S) inopinatus*, once these invertebrates are intermediate hosts to this nematode species (Hoshino & Tavares-Dias, 2014), with *C. amazonarum* as the definitive host. Low parasitism by *P. (S) inopinatus* during the dry and rainy seasons probably resulted from the environmental fluctuations in the Igarapé Fortaleza basin, which did not favor their transmission process, since this nematode has a short life cycle. Leeches were found at low levels of parasitism on the gills of *C. amazonarum* and only in the rainy season. Similar findings have also been reported for *Hoplias malabaricus* from the Brazilian Amazon (Gonçalves et al., 2016).

The length-weight relationship in *C. amazonarum* showed that weight did not increase proportionately to the increase in the length of the host; body size explained less than 35% of variance on parasite abundance. In addition, the condition factor was higher during the dry season. However, the *C. amazonarum* weight was higher during the rainy season, when the fish had greater access to food containing infective stages of the endoparasites. This thus indicates that fish with greater weights can tolerate higher levels of parasitism. In contrast, the seasonal variation of endoparasites and ectoparasites did not affect the condition factor of other fish populations of the eastern Amazon (Gonçalves et al., 2016; Tavares-Dias et al., 2014). Such differences between hosts in response to the condition factor may be attributed to different transmission strategies used by the different parasites (Gonçalves et al., 2016).

In summary, this first study on parasites of wild *C. amazonarum* populations indicated that the Amazonian hydrological cycle, host feeding behavior, and seasonal availability of the infectious stages of the parasites were determining factors for seasonal variations of the endoparasites. Furthermore, this study corroborates previous observations that some parasites undergo changes relating to seasonality, while others do not. For this wild fish population, a study on the seasonal dynamics of the parasite community might provide supporting knowledge for use in fishing and fish farming. This study may be of help in the holding facilities of this ornamental fish, to better

understand the parasites dynamics. Finally, this information is useful for preventive programs involved with disease control and in indicating the correct season for applying prophylactic measures to reduce the negative impact(s) of parasites in captured fish.

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