

Seasonal pattern in parasite infracommunities of *Hoplerythrinus unitaeniatus* and *Hoplias malabaricus* (Actinopterygii: Erythrinidae) from the Brazilian Amazon

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Abstract

The present study investigated the effects of seasonal variation in parasites infracommunities of *Hoplerythrinus unitaeniatus* and *Hoplias malabaricus* from a tributary of Amazon River. For *H. unitaeniatus* and *H. malabaricus*, 11 parasite species were similar, and greatest parasite richness occurred during the rainy season. *Ichthyophthirius multifiliis* was the dominant parasite species for both hosts. In *H. unitaeniatus*, infection by *Whittingtonocotyle caetei*, *Whittingtonocotyle jeju*, *Urocleidoides* sp. and *Anacanthorus* sp. was higher during rainy season. *Contraecaecum* sp., *Procamallanus* (*Spirocamallanus*) *inopinatus*, *Nomimoscolex matogrossensis* and *Gorytocephalus spectabilis* showed no seasonal pattern. For *H. unitaeniatus*, *P. pillulare*, *Clinostomum marginatum* and *Argulus pestifer* occurred only during dry season, while *Trichodina* sp., *Dolops geayi*, undetermined metacercariae and *Posthodiplostomum* sp. occurred only during the rainy season. In *H. malabaricus*, the prevalence of *Urocleidoides eremitus* was similar during the two seasons, but abundance was higher during the rainy season. *Tetrahymena* sp., *C. marginatum*, *Dendrorchis neivai*, undetermined metacercariae, *Posthodiplostomum* sp., *Genarchella genarchella*, *Cystidicoloides* sp., *G. spectabilis*, *D. geayi*, *A. pestifer* and Glossiphoniidae gen. sp. occurred only during the dry season. However, *Contraecaecum* sp. and *P. (S.) inopinatus* occurred during both seasons, but the prevalence of *P. (S.) inopinatus* was higher during the rainy season. Seasonal variation in this infection levels was due to the host's feeding behavior and habits and the availability of infectious forms of parasites with heteroxenic life cycles. The non-seasonal fluctuation detected are likely a result of the parasites biology, highly variable nature of this tributary of Amazon River and low abundance of parasites.

Keywords

Amazon, Erythrinid, parasites, seasonal variation

Introduction

Hoplerythrinus unitaeniatus Spix and Agassiz, 1829 (Jeju or Aimara) and *Hoplias malabaricus* Bloch, 1794 (Thraira) are fish in the order Characiformes and family Erythrinidae, with wide distribution in South and Central America. These benthopelagic species are sedentary and occur in several types of fluvial and lacustrine environments, especially in shallow water environments and near submerged or marginal vegetation. They are piscivorous fish when adults, but juveniles also feed on plankton, crustaceans, insects and seeds. These species are habitually active at twilight and night and do not undertake migration (Santos *et al.* 2006; Soares *et al.* 2011; Alcântara and Tavares-Dias 2015). They are also commonly found in the Amazon River system, which is a complex ecosystem that in-

cludes tributary rivers and streams, with floodplains and periodically flooded forest alongside them.

In South America, the parasites fauna of *H. malabaricus* has been constituted by species of Protozoa, Isopoda, Monogenoidea, Cestoda, Nematoda and Digenea. The parasites fauna of *H. unitaeniatus* has been comprised species of Protozoa, Argulidae, Monogenoidea, Cestoda, Nematoda, Acanthocephala and Digenea (Chambrier *et al.*, 1996; Rocha 2011; Thatcher 2006; Alcântara and Tavares-Dias 2015). Parasite abundance and species richness have not been found to be affected by host body size, therefore indicating that factors other than host body size are more important determinants of variation of parasite abundance and species richness for the populations of both of these hosts Erythrinidae (Alcântara and Tavares-Dias 2015). One of the factors structuring these

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parasite communities in *H. malabaricus* and *H. unitaeniatus* may be the dry and rainy cycles from the eastern Amazon region. Therefore, can the parasites of *H. unitaeniatus* and *H. malabaricus* to suffer also seasonal influence?

In the eastern Amazon region, the tributaries of the Amazon River not only are strongly influenced by river tides, but also are influenced by the local rainfall regime (Takiyama *et al.* 2012; Tavares-Dias *et al.* 2014). Thus, seasonality in the Amazon basin is divided into a rainy season and a dry season. This dry/rainy season cycle may influence the life of the aquatic organisms and parasite infection levels in host fish (Kadlec *et al.* 2003; Thatcher 2006; Vital *et al.* 2011; Neves *et al.* 2013; Tavares-Dias *et al.* 2014). This knowledge provides information on different parasite species functions within a community, and also whether these parasitism levels are constant or whether variations are influenced by intermediate host availability. Such variations can alter the recruitment process of parasite species in the environment (Valtonen *et al.* 1990; Kadlec *et al.* 2003; Violante-González *et al.* 2008; Neves *et al.* 2013; Soylu 2013; Muñoz *et al.*, 2013; Tavares-Dias *et al.* 2014). Therefore, the effects of seasonality on the parasite infracommunities in wild *H. unitaeniatus* and *H. malabaricus* populations in the Amazon region need to be addressed. Thus, this study investigated the effects of the rainy and dry seasons on parasite infracommunities in *H. unitaeniatus* and *H. malabaricus* from the eastern Amazon region, Northern Brazil.

Material and Methods

Fish and collection locality

Between February and December 2013, 69 specimens of *H. unitaeniatus* (22.3 ± 2.3 cm and 150.6 ± 40.6 g) and 67 specimens of *H. malabaricus* (23.5 ± 4.4 cm and 164.9 ± 85.8 g) were collected in the Igarapé Fortaleza basin ($00^{\circ}00'56.3$ N, $051^{\circ}05'27.1$ W), a tributary of the Amazon River system, near the city of Macapá in the state of Amapá (Brazil), for parasitological analysis. All the fish were juveniles and adults not sexed, and were collected using fishing nets of different mesh sizes (20–50 mm) and transported packed in ice to the Aquatic Organism Health Laboratory from Embrapa Amapá, Macapá (AP).

In the eastern Amazon region of Brazil, there is a tropical climate region, and the rainy period occurs from December to May and the dry period from June to November (Souza and Cunha 2010). Seasonality was therefore based on the rainy season and dry season. Rainfall data were obtained from the Hydrometeorology and Renewable Energy Center (NHMET) of the Institute of Scientific and Technological Research of the State of Amapá (IEPA).

Parasite collection procedures and data analysis

The fish were necropsied and the mouth, opercula and gills were examined to detect ectoparasites. Gills were collected

and fixed in formalin 5%. Following this, all the entrails and the gastrointestinal tract were also examined to detect endoparasites using a stereoscopic microscope. They were collected, fixed in formalin 5%, preserved in 70% ethyl alcohol, clarified or stained for identification (Eiras *et al.* 2006; Thatcher 2006). The identification of parasite taxa was based on specialized literature (Moravec 1998; Malta 1982; Chambrier *et al.* 1996; Thatcher 2006).

To analyze the parasite infracommunities, prevalence and abundance were taken into consideration, as recommended by Bush *et al.* (1997). The following descriptors for the parasite community were calculated: (1) species richness; (2) Brillouin diversity index (*HB*); (3) evenness (*E*) in association with diversity index; and (4) Berger-Parker dominance index (*d*) and dominance frequency (percentage of the infracommunities in which a parasite species is numerically dominant (Rohde *et al.* 1995; Magurran 2004), using the Diversity software (Pisces Conservation Ltd., UK).

The Shapiro-Wilk test was used to determine whether the parasite abundance data followed a normal distribution pattern. Differences in parasite prevalence between rainy and dry periods and seasons were evaluated using the chi-square test (χ^2) and differences in parasite abundance were evaluated using the Mann-Whitney test (*U*). For each host, the differences in species richness, diversity (*HB*), (*E*) and dominance (*d*), between rainy and dry periods and between host species, were determined using the Mann-Whitney test (*U*) (Zar 2010). Seasonal comparisons of parasite prevalence and abundance were done only for parasites with prevalence >10%.

Body weight (g) and total length (cm) data were used to calculate the relative condition factor (*Kn*) of the hosts, compared with the standard value (*Kn* = 1.0) (Le-Cren 1951). The values were described as the mean followed by standard deviation (\pm SD) and range (minimum and maximum).

Differences in host *Kn* values, between the rainy and dry seasons, were compared using the Mann-Whitney test (*U*). Correlations between host length and the species richness and Brillouin index were performed using Spearman's correlation coefficient (*rs*) (Zar 2010).

Results

During the rainy season, the mean water temperature was $29.6 \pm 0.3^{\circ}\text{C}$; pH was 6.1 ± 0.4 ; dissolved oxygen was 2.6 ± 0.3 mg L⁻¹ and rainfall was 362.2 ± 136.1 mm. During the dry season, the mean water temperature was $30.5 \pm 0.3^{\circ}\text{C}$; pH was 6.3 ± 0.3 ; dissolved oxygen was 2.0 ± 0.3 mg L⁻¹ and rainfall was 87.9 ± 62.8 mm.

Parasite infracommunities in *Hoplerthrinus unitaeniatus*

All the fish examined were parasitized during both seasons, and a total of 1,262,019 parasites were collected during the dry season and 76,460 parasites during the rainy season. The parasite

Table 1. Seasonal variation of the prevalence (P) and mean abundance (MA) ± SD of parasites in *Hoplerhythrinus unitaeniatus* from Brazilian Amazon. SI: Site of infection, U = Mann-Whitney test, c2= chi-square test., *p< 0.05, **p< 0.001

Parasite species	SI	Dry season (n = 30)			Rainy season (n = 39)			χ ²	U
		P (%)	MA	P (%)	P (%)	MA			
<i>Ichthyophthirius multifiliis</i>	Gills	100	41.094.2 ± 27.465	97.4	1.923.1 ± 2.544.7	0.781	3.00**		
<i>Piscinoodinium pillulare</i>	Gills	83.3	960.7 ± 1.026.1	0	0	-	-		
<i>Trichodina</i> sp.	Gills	0	0	5.1	13.2 ± 75.2	-	-		
<i>Whittingtonocotyle caetei</i> and <i>Whittingtonocotyle jeju</i>	Gills	53.3	1.2 ± 1.6	92.3	8.1 ± 10.9	13.872**	946.5**		
<i>Urocleidoides</i> sp.	Gills	16.7	0.4 ± 1.5	76.9	6.1 ± 8.1	24.632**	944.5**		
<i>Anacanthorus</i> sp.	Gills	10.0	0.3 ± 1.0	66.7	4.2 ± 6.3	22.348**	926.0**		
Encysted metacercariae	Caecum	0	0	2.6	0.1 ± 0.3	-	-		
<i>Clinostomum marginatum</i> (metacercariae)	Gills, intestine	40.0	2.2 ±	0	0	-	-		
<i>Posthodiplostomum</i> sp. (metacercariae)	Abdominal cavity, gills, intestine	-	-	30.8	1.6 ± 0.9	-	-		
<i>Contracaecum</i> sp. (larvae)	Liver, intestine, caecum	76.7	5.8 ± 9.7	74.4	3.7 ± 3.2	0.049	495.5		
<i>Procammallanus</i> (<i>S.</i>) <i>inopinatus</i> (adults)	Abdominal cavity, caecum, stomach	23.3	1.1 ± 2.9	41.0	1.6 ± 1.0	2.388	511.0		
<i>Nomimoscolex matogrossensis</i> (plerocercoids)	Abdominal cavity, stomach	13.3	0.2 ± 0.5	20.5	2.3 ± 1.3	1.012	540.0		
<i>Gorytocephalus spectabilis</i> (cistacants and adults)	Stomach, intestine	36.6	1.2 ± 2.0	41.0	3.6 ± 3.6	0.135	576.5		
<i>Dolops geayi</i>	Gills	0	0	2.6	0.03 ± 0.2	-	-		
<i>Argulus pestifer</i>	Gills	6.7	0.1 ± 0.2	0	0	-	-		

Table II. Differences in diversity indices between seasons for parasites collected from *Hoplerythrinus unitaeniatus* from Brazilian Amazon. U: Mann–Whitney test

Diversity indices	Dry season (n = 30)	Rainy season (n = 39)	U	p
Species richness	4.7 ± 1.5 (2–9)	5.5 ± 1.4 (3–8)	793.5	0.005
Brillouin (HB)	0.13 ± 0.08 (0.003–0.304)	0.16 ± 0.21 (0.006–1.080)	520.0	0.435
Evenness (E)	0.05 ± 0.03 (0.01–0.123)	0.08 ± 0.12 (0.002–0.627)	538.0	0.738
Berger–Parker (d)	0.97 ± 0.02 (0.91–1.00)	0.94 ± 0.14 (0.30–1.00)	678.0	0.263

community of *H. unitaeniatus* consisted of species of protozoa, monogeneans, digeneans, nematodes, cestodes, acanthocephalans and crustaceans. *Ichthyophthirius multifiliis* Fouquet, 1876 (Ichthyophthiriidae), was the most prevalent and abundant parasite in both seasons and showed higher abundance during the dry season. Infection due to *Piscinoodinium pillulare* Schäperclaus, 1954, Lom, 1981 (Oodiniaceae) and *Argulus pestifer* Ringuélet, 1948 (Argulidae) only occurred during the dry season. However, *Trichodina* sp.; *Dolops geayi* Bouvier, 1897 (Argulidae); undetermined digenean larvae and *Posthodiplostomum* sp. occurred only during the rainy season. Moreover, in the gills, the prevalence and abundance of monogeneans *Whittingtonocotyle caetei* Santos-Neto, Rodrigues & Domingues, 2015; *Whittingtonocotyle jeju* Santos-Neto, Rodrigues et Domingues, 2015; *Urocleidoides* sp. and *Anacanthorus* sp. (Dactylogyridae) were higher during the rainy season. *Argulus pestifer* occurred only during the dry season while *D. geayi* occurred only during the rainy season. The prevalence and abundance of *Contracaecum* sp., *Procamallanus (Spirocamallanus) inopinatus* Travassos, Artigas et Pereira, 1928 (Camallanidae), *Nomimoscolex matogrossensis* Rego et Pavanelli, 1990 (Monticellidae) and *Gorytocephalus spectabilis* Machado, 1959 (Neoechinorhynchidae) were similar during the rainy and dry seasons. However, *Clinostomum marginatum* Rudolphi, 1819 (Clinostomidae) occurred only during the dry season while *Posthodiplostomum* sp. occurred only during the rainy season (Table I).

For *H. unitaeniatus*, *I. multifiliis* was dominant (dominance frequency, DF = 0.970) during the dry season, followed by *P. pillulare* (DF = 0.022); during the rainy season, *I. multifiliis* was also dominant (DF = 0.980), followed by *Trichodina* sp. (DF = 0.006).

In *H. unitaeniatus*, species richness was higher during the rainy season, but the Brillouin index, evenness and Berger–Parker dominance did not differ between the dry and rainy seasons (Table II). During the dry season, hosts infected by 3 to 5 parasite species predominated, but during the rainy season, they presented 4 to 6 parasite species (Fig. 1)

The *H. unitaeniatus* growth during the dry season ($b = 1.367$; $r^2 = 0.761$) and rainy season ($b = 1.532$; $r^2 = 0.899$) was negative allometric, indicating greater increase in body weight than in size. Total Kn did not differ from the standard Kn = 1.00 ($U = 2244.0$; $p = 0.753$) and also did not differ between the dry and rainy seasons (Fig. 2), thus indicating good body conditions. The host weight during the dry season (148.1 ±

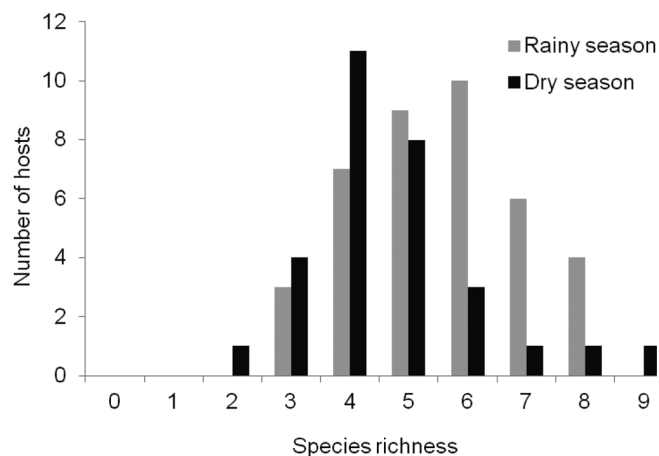


Fig. 1. Species richness of parasites during dry and rainy season for *Hoplerythrinus unitaeniatus* from Brazilian Amazon

42.8 g) was similar ($t = -0.509$; $p = 0.614$) to that of the rainy season (152.4 ± 43.5 g). The host length during the dry season (21.9 ± 2.5 cm) was also similar ($t = -1.154$; $p = 0.258$) to than host length during the rainy season (22.6 ± 2.0 cm). Host length did not show any correlation with parasite species richness during the dry season ($r_s = -0.041$; $p = 0.829$) and rainy season ($r_s = 0.064$; $p = 0.697$), and neither did the Brillouin diversity during the dry season ($r_s = 0.212$; $p = 0.261$) and rainy season ($r_s = -0.081$; $p = 0.624$).

Parasite infracommunities in *Hoplias malabaricus*

All the fish were parasitized during both seasons and a total of 433,656 parasites were collected during the dry season and 206,935 parasites during the rainy season. The parasite community consisted of species of protozoa, monogeneans, digeneans, nematodes, cestodes, acanthocephalans, crustaceans and hirudineans. The ciliate *I. multifiliis* was the most prevalent and abundant parasite during both seasons and without any difference in infection levels between the seasons. The prevalence and abundance of *P. pillulare* were higher during the dry season. The prevalence of *Urocleidoides eremitus* Kritsky, Thatcher et Boeger, 1986 (Dactylogyridae) was similar during the two seasons, but its abundance was higher during the rainy season. *Tetrahymena* sp., *N. matogrossensis*, *C. marginatum*, *Dendrorchis neivai* Travassos, 1926, *Posthodiplostom-*

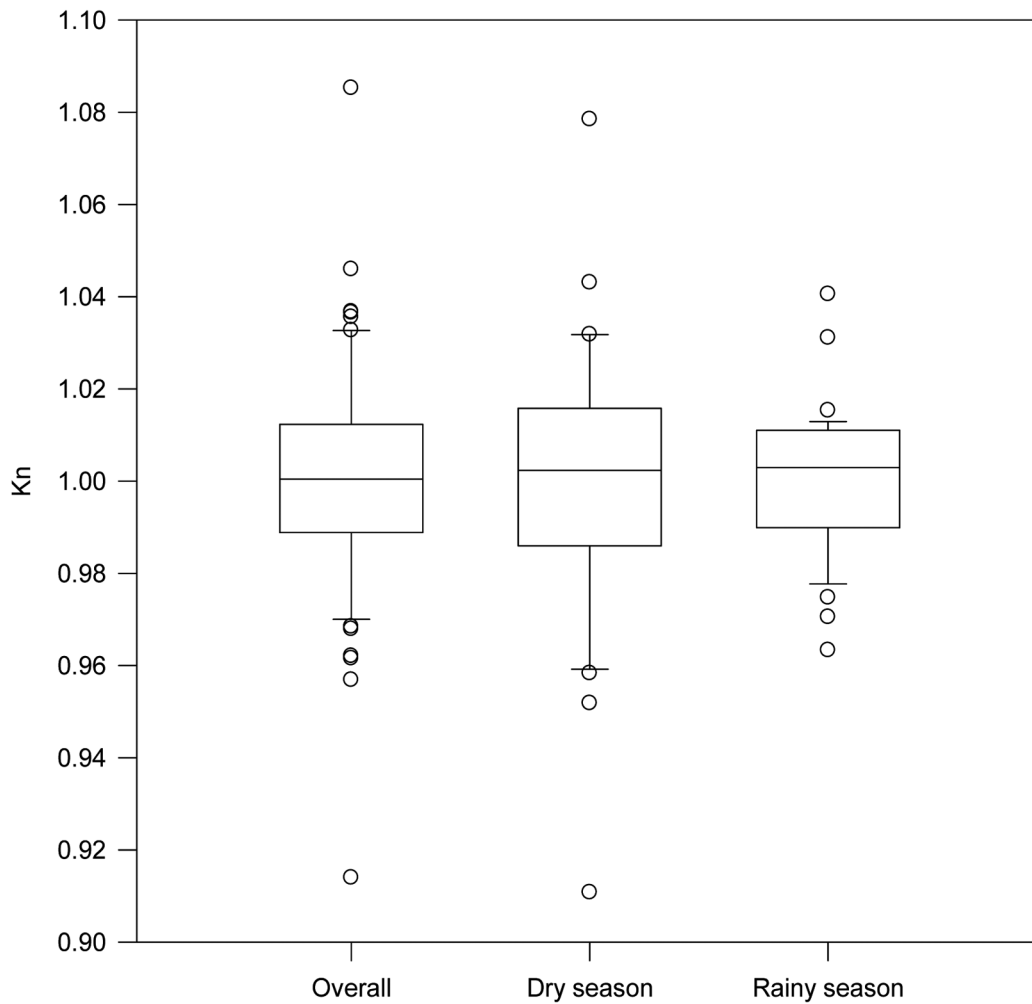


Fig. 2. Relative condition factor (K_n) of *Hoplerythrinus unitaeniatus* collected in the Brazilian Amazon. Box plots represent medians, interquartile ranges, minimum–maximum ranges and outliers. Equal mean values according to the Kruskal–Wallis test ($p = 0.879$)

mum sp., *Genarchella genarchella* Kohn et Fernandes, 1988, undetermined digenean larvae and *G. spectabilis* occurred only during the dry season. However, leeches Glossiphoniidae, *Cystidicoloides* sp., *D. geayi* and *A. pestifer* occurred only during the rainy season, while *Contracaecum* sp. and *P. (S.) inopinatus* occurred during both seasons, but the prevalence of *P. (S.) inopinatus* was higher in rainy season (Table III).

For *H. malabaricus*, *I. multifiliis* was also dominant in the dry season (dominance frequency, $DF = 0.934$), followed by *P. pillulare* ($DF = 0.060$) and *U. eremitus* ($DF = 0.002$); during the rainy season, *I. multifiliis* was also dominant ($DF = 0.949$), followed by *U. eremitus* ($DF = 0.030$).

In *H. malabaricus*, there was no difference in the Brillouin index, evenness or Berger-Parker dominance index between the dry and rainy seasons. However, parasite species richness was higher during the rainy season (Table IV). During the dry season, there was predominance of hosts infected by 3 to 5 parasites, but during rainy season, the hosts presented 5 to 7 parasites (Fig. 3).

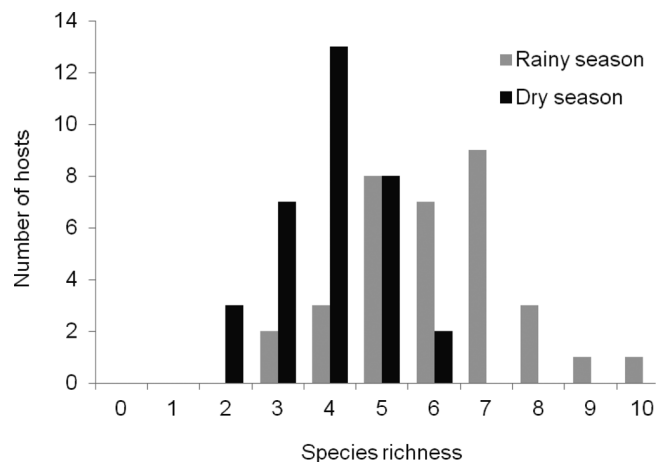


Fig. 3. Species richness of parasites during dry and rainy season for *Hoplias malabaricus* from Brazilian Amazon

Table III. Seasonal variation of the prevalence (P) and mean abundance (MA) \pm SD of parasites in *Hoplias malabaricus* from Brazilian Amazon. SI: Site of infection, U = Mann–Whitney test, χ^2 = chi-square test, *p < 0.05, **p < 0.001

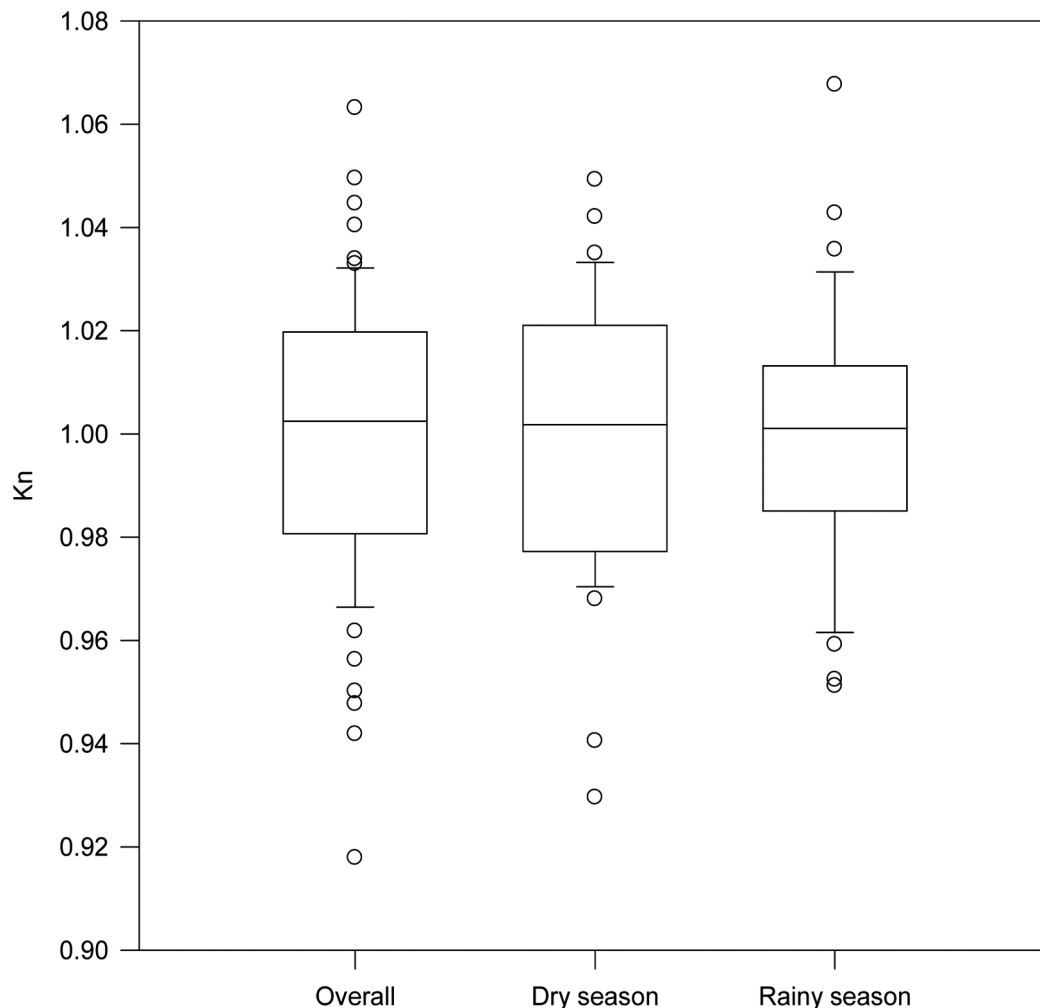
Parasite species	SI	Dry season (n = 33)			Rainy season (n = 34)			χ^2	U
		P (%)	MA	P (%)	MA	P (%)	MA		
<i>Ichthyophthirius multifiliis</i>	Gills	100	12.278.7 \pm 13.034.6	100	5.780.6 \pm 5.127.2	0	464.0		
<i>Piscinoodinium pillulare</i>	Gills	51.5	795.5 \pm 1.722.5	11.8	15.8 \pm 56.7	12.296**	306.00**		
<i>Tetrahymena</i> sp.	Gills	12.1	25.5 \pm 115.0	0	0	–	–		
<i>Urocleidoides eremitus</i>	Gills	97.0	28.2 \pm 28.4	100	195.7 \pm 157.3	1.046	999.0**		
<i>Clinostomum marginatum</i> (metacercariae)	Gills, intestine, stomach	30.3	1.3 \pm 3.2	–	–	–	–		
<i>Dendrorchis neivai</i>	Abdominal, intestine cavity	29.4	16.8 \pm 60.3	–	–	–	–		
Encysted metacercariae	Liver, intestine	88.2	32.1 \pm 64.9	–	–	–	–		
<i>Posthodiplostomum</i> sp. (metacercariae)	Gills, intestine, ovary	32.4	6.9 \pm 32.5	–	–	–	–		
<i>Genarchella genarchella</i> (metacercariae)	Gills, stomach, caecum	32.4	25.6 \pm 60.4	–	–	–	–		
<i>Cystidicoloides</i> sp. (larvae)	Stomach	0	0	17.6	0.6 \pm 1.5	–	–		
<i>Contracaecum</i> sp. (larvae)	Stomach, liver, intestine, ovary, abdominal cavity Caecum	69.7	11.2 \pm 15.3	70.6	7.5 \pm 10.7	0.006	505.5		
<i>Procammallanus</i> (<i>S.</i>) <i>inopinatus</i> (larvae and adults)	Intestine, abdomen cavity, caecum, stomach	9.1	0.1 \pm 0.4	32.4	1.0 \pm 2.4	5.482*	428.0		
<i>Gorytocephalus spectabilis</i> (cistacants and adults)	Intestine, abdominal cavity, Caecum	20.6	0.8 \pm 2.2	–	–	–	–		
<i>Nomimoscolex matogrossensis</i> (plerocercoids)	Intestine	21.2	0.7 \pm 1.7	0	0	–	–		
<i>Dolops geayi</i>	Gills	0	0	14.7	0.3 \pm 0.7	–	–		
<i>Argulus pestifer</i>	Gills	0	0	5.9	0.1 \pm 0.2	–	–		
Glossiphoniidae gen. sp.	Gills	0	0	5.9	0.1 \pm 0.2	–	–		

Table IV. Differences in diversity indices between seasons for parasites collected of *Hoplias malabaricus* from Brazilian Amazon. *U*: Mann–Whitney test

Diversity indices	Dry season (n = 33)	Rainy season (n = 34)	<i>U</i>	<i>p</i>
Species richness	3.9 ± 1.0 (2–6)	6.0 ± 1.5 (3–10)	964.5	0.001
Brillouin (<i>HB</i>)	0.25 ± 0.25 (0.001–0.80)	0.33 ± 0.30 (0.009–1,31)	676.0	0.151
Evenness (<i>E</i>)	0.11 ± 0.11 (0.0006–0.36)	0.12 ± 0.11 (0.003–0.49)	630.0	0.390
Berger–Parker (<i>d</i>)	0.91 ± 0.11 (0.61–0.99)	0.89 ± 0.13 (0.38–0.99)	465.0	0.231

The *H. malabaricus* growth during the dry season ($b = 2.175$; $r^2 = 0.934$) and rainy season ($b = 1.978$; $r^2 = 0.944$) was negative allometric, indicating that there was a greater increase in body weight than in size. Total Kn did not differ from the standard $Kn = 1.0$ ($U = 2077.0$; $p = 0.427$) and also did not differ between the dry and rainy seasons (Fig. 4), thus indicating good body conditions, although host weight during the dry season (113.8 ± 43.7 g) was significantly lower ($U = 979.0$; $p =$

0.001) than during the rainy season (214.4 ± 87.9 g). Host length during the dry season (20.9 ± 3.0 cm) was also significantly lower ($t = -6.132$; $p = 0.001$) than host length during the rainy season (26.0 ± 0.6 cm). Host length did not show any correlation with parasite species richness during the dry season ($rs = 0.196$; $p = 0.275$) and rainy season ($rs = -0.161$; $p = 0.362$), just as Brillouin diversity during dry season ($rs = 0.168$; $p = 0.349$) and rainy season ($rs = -0.083$, $p = 0.638$).

**Fig. 4.** Relative condition factor (*Kn*) of *Hoplias malabaricus* collected in the Brazilian Amazon. Box plots represent medians, interquartile ranges, minimum–maximum ranges and outliers. Equal mean values according to the Kruskal–Wallis test ($p = 0.977$)

Discussion

The *H. unitaeniatus* parasite community consisted of 3 species of protozoans and 12 of metazoans, of which seven were endoparasites and mostly in larval form. The *H. malabaricus* community consisted of 3 species of protozoans and 15 of metazoans, of which 11 were endoparasites species, mostly also in larval form. However, for both host species, 11 parasite species were common, and *I. multifiliis* was the dominant species. *Ichthyophthirius multifiliis* is an ectoparasite species with direct life cycle and great capacity for reproduction in eutrophic environments (Neves *et al.* 2013; Tavares-Dias *et al.* 2014; Majumder *et al.* 2015), such as the environment of the present study. The quantity of rainfall may have a great effect on aquatic ecosystems, resulting in changes to fish habitat and water velocity, which may influence fish life and therefore may affect the composition of the parasite communities and intermediate hosts. It may also lead to an increase in the physiological stress on the host fish and therefore make them more susceptible to parasite infections (Kadlec *et al.* 2003; Tavares-Dias *et al.* 2014). Both of the carnivorous fish, *H. malabaricus* and *H. unitaeniatus*, are benthopelagic and sedentary species that live in environments of stagnant or slowly running water and near submerged or marginal vegetation (Santos *et al.* 2006; Soares *et al.* 2011; Alcântara and Tavares-Dias 2015). They present higher endoparasite richness, with higher presence of the larval stage. Therefore, parasite assemblages could also play a potential role as environmental indicators by decreasing or increasing in their diversity, species richness, abundance and prevalence with the changes to environmental conditions (Kadlec *et al.* 2003; Tavares-Dias *et al.* 2014; Alcântara and Tavares-Dias 2015).

In wild fish populations, the endoparasite community structuring is related to host behavior, position in the food chain, seasonal availability of infecting forms in the environment and environmental conditions (Kadlec *et al.* 2003; Violante-González *et al.* 2008; Soylu 2013; Neves *et al.* 2013; Tavares-Dias *et al.* 2014; Majumder *et al.* 2015). Therefore, the similar lifestyles of *H. malabaricus* and *H. unitaeniatus* and their carnivorous habits, feeding on fish, crustaceans (Santos *et al.* 2006; Soares *et al.* 2011; Alcântara and Tavares-Dias 2015) and mollusks, contributed towards the elevated richness of endoparasites, which are transmitted trophically. In fish living in tropical fresh water, the highest endoparasite richness is generally observed in hosts with a diet based on macroinvertebrates and fish (Violante-González *et al.* 2008).

Seasonal variations may be reflected in parasite populations; hence, it can be expected that ectoparasites in particular, which have direct ties to the external environment, would not remain indifferent to the dry/rainy cycle changes (Neves *et al.* 2013; Tavares-Dias *et al.* 2014). In *H. malabaricus* and *H. unitaeniatus*, infections by *I. multifiliis* occurred in a similar manner during the rainy and dry seasons, but in *H. unitaeniatus* there was higher abundance during the dry season. *Piscinoodinium pillulare* occurred in the gills of

H. malabaricus during both seasons, but showed higher prevalence and abundance during the dry season. However, in *H. unitaeniatus*, infection by *P. pillulare* occurred only during the dry season, when there is a reduction of the environment in the basin studied here. However, low levels of infection by *Trichodina* sp. was found only in *H. unitaeniatus* and during the rainy season, while *Tetrahymena* sp. occurred only in *H. malabaricus* and during the dry season. The basin investigated here is strongly influenced by high loads of organic material from the Amazon River, and by local urban eutrophication (Tavares-Dias *et al.* 2014). The reduction of rainfall levels during the dry season increases this eutrophication, thereby favoring reproduction of these protozoans.

Due to seasonal changes that are reflected in parasite populations, monogenoidean species may present a negative or positive response to these changes, since these ectoparasites have a strong direct relationship with the external environment. Water temperature is the greatest factor influencing the seasonal occurrence of monogenoideans in temperate climatic regions. Variations in monogenoidean population size are strongly influenced by seasonal changes in environmental temperature, which directly affects the reproduction, survival time and behavior of these ectoparasites, as well as the behavior, density and responses of the hosts to infection levels. Thus, while some of them tend to reproduce more at a higher water temperature, others prefer a cool water temperature (Chubb, 1977; Valtonen *et al.* 1990; Soylu 2013). In contrast, in the Amazon region, the infection levels of these ectoparasites are strongly related to the hydrological cycle (Vital *et al.* 2011; Neves *et al.* 2013; Tavares-Dias *et al.* 2014).

In *H. unitaeniatus* and *H. malabaricus* there was infection by the monogenoideans *W. caetei*, *W. jeju*, *U. eremitus*, *Urocleidoides* sp. and *Anacanthorus* sp. during both seasons, but higher levels occurred during the rainy season. Therefore, these results indicate that reproduction among these dactylogyrids follow a well-defined pattern, i.e. with high prevalence and abundance throughout the year but with the highest peak of abundance during the rainy season. In contrast, higher infection levels by *Gussevia asota* Kritsky, Thatcher et Boeger, 1989, *Gussevia astronoti* Kritsky, Thatcher et Boeger, 1989 and *Gussevia rogersi* Kritsky, Thatcher et Boeger, 1989 in *Astronotus ocellatus* Agassiz, 1831 (Neves *et al.* 2013), and also by *Gussevia alioides* Kritsky, Thatcher et Boeger, 1986 and *Gussevia disparoides* Kritsky, Thatcher et Boeger, 1986 in *Aequidens tetramerus* Heckel, 1840 (Tavares-Dias *et al.* 2014), which occurred during the dry season in the eastern Amazon region. According Chubb (1977), seasonal differences between or within the different genera of monogenoideans are known to be related to their direct life cycle. Moreover, these results suggest that in the Amazon region, which has a similar temperature, the dactylogyrids species has developed a life strategy of its own in order to take advantage the dry/rainy season cycle. On the other hand, in temperate climatic regions, the temperature is the factor that directly affects the reproduction of dactylogyrids and the

host response to parasitic infection (Chubb 1977; Valtonen *et al.* 1990; Kadlec *et al.* 2003).

In Brazil, the mollusks *Biomphalaria* spp. are the primary intermediate host of *C. marginatum*, several fish species are the secondary intermediate hosts and several fish-eating birds are the definitive hosts. Similarly, bivalve mollusks are the primary intermediate host of *Posthodiplostomum nanum* Dubois, 1937, fish species are the secondary intermediate hosts and fish-eating birds are the definitive hosts (Pinto and Melo 2012; Pinto *et al.* 2013; Pinto *et al.* 2015). Infections of *C. marginatum* occurred only in the dry season in *H. malabaricus* and *H. unitaeniatus* while infections due to *Posthodiplostomum* sp. occurred during the rainy season in *H. unitaeniatus* and dry season in *H. malabaricus*. However, in *H. malabaricus*, the undetermined digenean larvae, *D. neivai* and *G. genarchella*, occurred only in the dry season. These results showed that a defined transmission pattern exists for the infectious forms of these digenean species for these two hosts, but with low overlapping of habitats. Seasonal variations in the abundance of intermediate hosts (mollusks) and definitive hosts (aquatic birds) can influence the availability of the digenean species (Chubb, 1979; Soylu 2013). During the dry season in the Amazon region, there is greater host density and greater overlap of the intermediate and definitive hosts in a shrunken environment, thereby facilitating the transmission of these digenean species (Tavares-Dias *et al.* 2014), which may be either ectoparasites or endoparasites of *H. unitaeniatus* and *H. malabaricus*.

Studies focusing on directly transmission of parasites have also reported a relationship between host abundance and the abundance of digeneans, both influenced by the dry/rainy season cycle (Violante-González *et al.* 2008; Muñoz *et al.*, 2013). Moreover, environmental variations (Violante-González *et al.* 2008; Muñoz *et al.*, 2013), vegetation cover and seasonal variations relating to the dietary behavior of *H. malabaricus* and *H. unitaeniatus* may also influence the seasonal infection patterns of metacercariae. These two host fish are piscivorous when adults, while the juvenile fish feeds on plankton, crustaceans, insects and seeds (Santos *et al.* 2006; Soares *et al.* 2011; Alcântara and Tavares-Dias 2015) and mollusks. This is the first record of *D. neivai* and *G. genarchella* for *H. malabaricus*.

In the basin of this study, there is greater food availability for the fish population during the rainy season (Gama and Halboth 2004). However, the foraging fish that form the intermediate hosts, along with crustaceans that contain some infectious forms of endoparasites, seemed to present greater food intake during the dry season. Other infectious forms of endoparasites presented availability in both seasons. For both *H. malabaricus* and *H. unitaeniatus*, infection due to *Contracaecum* sp. and *P. (S.) inopinatus* was not affected by seasonality. In contrast, for *A. ocellatus* (Neves *et al.* 2013) and *Pygocentrus nattereri* Kner, 1858 (Vital *et al.* 2011), there were higher levels of infection by these nematodes during the rainy season, due the seasonal dietary composition of these

hosts, which includes microcrustaceans that are intermediate hosts of nematodes (Chubb, 1980; Moravec 1998; Soylu 2013). *Cystidicoloides* sp. occurred only in *H. malabaricus* and also during the rainy season. This is the first record of *Cystidicoloides* sp. for *H. malabaricus*. Moreover, in *H. unitaeniatus*, infection by *G. spectabilis* was not affected by the dry and rainy season, while low levels of infection by this acanthocephalan in *H. malabaricus* was found only during the dry season. Similarly, for *A. tetramerus* from the Amazon River system, low levels of infection by *G. spectabilis* also occurred only in the dry season (Tavares-Dias *et al.* 2014). These results indicate that a differentiated seasonal pattern exists regarding encounters by *H. unitaeniatus* and *H. malabaricus* with intermediate hosts containing larvae and adults forms of *G. spectabilis*. Therefore, these two erythrinid species are definitive hosts of *G. spectabilis*, which also use other fish species as intermediate or definitive hosts, to complete their life cycle in this region.

In the wild fish population, the seasonal cycle may influence the cestodes parasite populations. Thus, the dynamics of the process of recruitment of plerocercoids in copepods and maintenance of plerocercoid populations in the intermediate and definitive hosts could vary with the seasonal cycle. Some plerocercoids of cestodes are present in all seasons, and therefore they may be invasive to certain definitive and intermediate hosts in all seasons or only during some seasons of the year (Chubb, 1980; Soylu 2013). *Diaptomus* or cyclopoid copepods serve as intermediate hosts for development of metacestodes or plerocercoids of Proteocephalidea species, which are ingested later on by a secondary intermediate host fish (Scholz 1999; Soylu 2013). Our results indicate that *H. unitaeniatus* and *H. malabaricus* are intermediate hosts for *N. matogrossensis*, in the environment of this study, because both fish species had a constant and moderate level of *N. matogrossensis* infection throughout both seasons, except that *H. malabaricus* was infected only in the dry season. This difference in seasonality may result from the biology of these host fish or from differences in their immune response. Moreover, these erythrinid species had also low overlapping of habitats. Chambrier *et al.* 1996) also reported low levels of *N. matogrossensis* in *H. malabaricus* from Paraguay, and attributed this to seasonal effects regarding occurrences of this proteocephalid species. However, these authors considered that this fish could be either a typical host or an accidental host for this cestode.

Infection by *D. geayi* in *H. malabaricus* and *H. unitaeniatus* was low and occurred only during the rainy season. High infection levels by *D. geayi* in *H. malabaricus* in the central Amazon was also found during the rainy season (Malta 1982). Low occurrence of *A. pestifer* in *H. unitaeniatus* was found only during the dry season, but in *H. malabaricus* this was during the rainy season, when the hydrological levels facilitated encounters between these argulids with hosts. In *Pseudoplatystoma* spp. was reported higher infection levels of *A. pestifer* during the dry season, since this argulid seems to prefer turbid waters, given that it does not have body pigmentation to

protect it from light that penetrates the water (Malta 1983). Another possible explanation for the seasonality of infection of these argulid species could be the effect of seasonal changes in local vegetation, and also the effects of tides of the Amazon River. Throughout the dry season, the aquatic vegetation facilitates proximity between *H. malabaricus*, *H. unitaeniatus* hosts and eggs of these argulids that are in vegetation. Moreover, lower rainfall levels mean that the tides of the Amazon River have less effect on the tributary river of this study, thereby facilitating encounters between these parasites and their hosts. However, these matters were not the focus of this investigation and therefore would need to be tested before any valid conclusions could be reached.

The condition factor can be used as a quantitative indicator for body conditions in fish populations in farmed and natural environmental situations (Guidelli *et al.* 2011). However, several factors may influence growth and the condition factor, including nutritional state, water temperature, stress, reproduction, parasitism and environmental alterations (Guidelli *et al.* 2011; Losee *et al.* 2014). *H. malabaricus* and *H. unitaeniatus* present both ectoparasites and endoparasites and the seasonality of these parasites does not affect the body conditions of these hosts, as also reported for *A. tetramerus* (Tavares-Dias *et al.* 2014). However, *Oncorhynchus kisutch* Walbaum, 1792 presents greater richness of endohelminths and showed better body conditions than fish with lower richness (Losee *et al.* 2014), thus indicating that fish with a higher condition factor can tolerate higher levels of parasitism. These differences in results may be related to different transmission strategies used by different parasites.

In summary, this first study on seasonality relating to *H. malabaricus* and *H. unitaeniatus* indicated that the Amazonian hydrological cycle, hosts' feeding behavior and seasonal availability of infectious forms in the environment were determining factors for seasonal variations of endoparasites infracommunities. However, the body size of the hosts was not an important factor in variations of species richness and diversity. Leeches and crustaceans were the least abundant species in the community structures of these two Amazonian hosts, which had high prevalence and abundance of generalist species such as *I. multifiliis* and *P. pillulare*. The two erythrinid species are fish with similar life histories and that inhabit the same environment; however, the difference (about 39%) in the parasites species found indicates that they had low overlapping of habitats, which was not expected. There were seasonal patterns for most of the parasite species; however, prolonged studies might indicate distinct annual patterns in parasite infracommunities relating to rainfall levels in different years. The non-seasonal fluctuation detected is likely to be a result of the parasites' biology, the highly variable nature of the Igarapé Fortaleza basin and the low abundance of parasites. Finally, for these natural fish populations, studies on the seasonal dynamics of parasite infracommunities and communities may provide supporting knowledge for use in fishing and fish farming.

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