

PARASITIC INFECTIONS IN TWO BENTHOPELAGIC FISH FROM AMAZON: THE AROWANA *Osteoglossum bicirrhosum* (Osteoglossidae) AND OSCAR *Astronotus ocellatus* (Cichlidae)

*INFECÇÕES PARASITÁRIAS EM DOIS PEIXES BENTOPELÁGICOS DA AMAZÔNIA: O ARUANÃ *Osteoglossum bicirrhosum* (Osteoglossidae) E APAIARI *Astronotus ocellatus* (Cichlidae)*

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ABSTRACT: The parasitic fauna of wild *Osteoglossum bicirrhosum* from central Amazon (Brazil) and wild *Astronotus ocellatus* from eastern Amazon (Brazil) as well as the host-parasite relationship were evaluated. 87.5% *O. bicirrhosum* were parasitized only by *Gonocleithrum aruanae* (Monogenoidea). 92.8% *A. ocellatus* were parasitized by *Ichthyophthirius multifiliis* (Ciliophora), *Gussevia asota* (Monogenoidea), *Posthodiplostomum* sp. metacercariae (Diplostomidae), *Procamallanus inopinatus* adults and *Contracaecum* larvae (Anisakidae). High levels of infection by *G. aruanae* occurred in *O. bicirrhosum* while in *A. ocellatus* the highest levels of infection were caused by helminthes ectoparasites such as *G. asota*, and endoparasites such as *Posthodiplostomum* sp., *P. inopinatus* and *Contracaecum* sp. There was a significant ($p > 0.05$) negative correlation between the intensity of helminthes species and the relative condition factor (Kn) from *A. ocellatus*, indicating that these parasites are pathogenic for hosts. The parasite fauna of *O. bicirrhosum* was scarce, while the parasites for *A. ocellatus* were mainly helminthes species, including adults and larval forms. Such differences on parasites fauna of both omnivore hosts may due to their history life as well as environmental and biological factors.

KEYWORDS: Condition Factor. Helminthes. Parasites. Freshwater Fish.

INTRODUCTION

Belonging to the Osteoglossidae family, the arowana *Osteoglossum bicirrhosum* Cuvier, 1829 occurs in the Amazon River Basin and in the Rupununi and Oyapock Rivers (FERRARIS-JUNIOR, 2003). A benthopelagic fish with a sedentary life-style, it can jump out of water to catch prey in nearby branches, which allows this species exploring a variety of arboreal preys, such as insects, arachnids and other small vertebrates. A small portion of its diet consists of small fish; it is an omnivorous species and also feeds on crustaceans and mollusks, showing no variations in the type of food from a hydrological period to another (SANTOS et al., 2006; AGUDELO-ZAMORA et al., 2007; SOARES et al., 2008). All these characteristics indicate that this is a carnivore-insectivore fish. Reproduction occurs between the end of the dry season (December) and the beginning of the flooding season (January). The females begin the process of sexual maturity at 55 cm out of standard length (SOARES et al., 2008).

The Oscar *Astronotus ocellatus* Agassiz, 1831 is a Cichlidae geographically distributed in South America, in the Amazonas River Basin,

including Brazil, Peru, Colombia and French Guiana (SANTOS et al., 2006; SOARES et al., 2008). However, it is currently found in river basins from the Northeast, Southeast and Southern Brazil (AZEVEDO et al., 2007). This species is widely used as food source by the Amazonian riverine and urban populations. Moreover, it is used as ornamental fish in several countries around the world (SILVA et al., 1993). It is a benthopelagic fish with preference for lentic environments finding protection under submerged branches and trunks. It can ambush and capture prey and escape over short distances; it is an omnivorous species, with carnivorous trend, feeding on fish, crustaceans, gastropods and larvae of aquatic insects (SANTOS et al., 2006; AZEVEDO et al., 2007; SOARES et al., 2008). Spawning occurs between the onset of flooding (December) and dry season (July) and the first gonadal maturation happens at 25 cm of length and 15-24 months of age (SOARES et al., 2008).

Despite the economic importance of *O. bicirrhosum* (ORTIZ; JOSÉ IANNACONE, 2008; COSTA et al., 2009) and *A. ocellatus* (AZEVEDO et al., 2007; SOARES et al., 2008) for aquarists, as well as source the importance as food for the riverine population from several areas of the

Amazon, a little is known about the parasitic fauna of these Amazon fish. Populations of arowana *O. bicirrhosum* from natural environments have been parasitized by the monogenoideans *Gonocleithrum aruanense*, *G. coenoideum*, *G. cursitans*, *G. planacroideum* and *G. planacrus* (KRITSKY; THATCHER, 1983), digeneans *Caballerotrema aruanense* (THATCHER, 1980; PELEGRINI et al., 2006), nematode *Camallanus acaudatus* (FERRAZ; THATCHER, 1990) and the pentastomida *Sebekia* sp. (PELEGRINI et al., 2006). In contrast, farmed arowana have been parasitized by *G. cursitans*, *Trichodina* sp. and *C. acaudatus* (VAZQUEZ et al., 2007). For *A. ocellatus* has been described parasitism by the monogenoideans *Gussevia asota*, *G. astronoti* and *G. rogersi* (KRITSKY et al., 1989; AZEVEDO et al., 2010; ABDALLAH et al., 2008), larvae of nematode *Contraecum* sp., crustacean *Lamproglana* sp., leeches *Placobdella* sp. and acanthocephalan *Polymorphus* sp., mollusks Glochidia larvae (AZEVEDO et al., 2007) and *Posthodiplostomum* sp. (TAVARES-DIAS et al., 2011).

In most aquatic ecosystems fish are hosts to parasites, which may affect the fish biology, mainly when they are intermediate hosts for larval parasites. Parasites may also alter the behavior of their hosts in order to increase their probability of survival, resulting in increased susceptibility of infecting the host to predation, enhancing the transmission by influence of predator-prey interactions (CHOUDHURY; COLE, 2008; BULLARD; OVERSTREET, 2008; POULIN; LEUNG, 2011). For these reason, the fish parasites may be used as indicators of environmental impacts.

The aim of this study was to evaluate the parasites fauna in wild populations of *O. bicirrhosum* from State of Amazonas (central Amazon) and of *A. ocellatus* from State of Amapá (eastern Amazon), Brazil. In addition, it provides an important baseline for comparative studies on host-parasite relationships in *O. bicirrhosum* and *A. ocellatus* from natural environments that may be much important for comparison on diseases studies regarding these fish when in fish farm, whose likelihood of infections can be higher and pathologies are devastating.

MATERIAL AND METHODS

Fish and study area

In October 2008, 28 specimens of *Osteoglossum bicirrhosum* (32-68 cm and 370-1.610 g) were collected from River Coari Grande (04°33'297"S, 64° 35'909"W), a tributary of the

middle Solimões, in the municipality of Coari, State of Amazonas (central Amazon), Brazil. In addition, from May to June 2009, 26 specimens of *Astronotus ocellatus* (19-26 cm and 192-470 g) were collected from River Preto (00°11'59.1"N, 051°32'89.3"W), a tributary of the Amazonas River, in the municipality of Mazagão, State of Amapá (eastern Amazon), Brazil, for parasitological studies. All fish were collected using different fishing nets (IBAMA: 11884-1).

Parasitological Analysis

All fish were weighed (g), measured in total length (cm) and then necropsy was performed. The gills were removed and analyzed under microscope. The gastro-intestinal tract was removed, placed in Petri dishes containing NaCl (0.8%) and examined under stereomicroscope. The methodology used for collection and fixation of parasites (EIRAS et al., 2006; Thatcher, 2006) and for their quantification (TAVARES-DIAS et al., 2001a, b) were according to the previous recommendations. The parasites identification was conducted according to KRITSKY et al. (1983), KRITSKY et al. (1989), MORAVEC (1998), THATCHER (2006) and LUQUE et al. (2011). The ecological terms adopted were those recommended by BUSH et al. (1997).

Parasite-host relationship

Data on weight and total length were used to determine the relative condition factor (Kn) of the fish (LE-CREN, 1951). Spearman's rank correlation coefficient (*rs*) was used to determine possible correlations between hosts' weight and length with the intensity of parasites (ZAR, 1999).

RESULTS

From the examined arowana *O. bicirrhosum*, 87.5% had their gills parasitized by *Gonocleithrum aruanense* Kristy & Thatcher, 1983 (Monogenoidea, Dactylogyridae), but no other parasite was found. For *A. ocellatus* from the Preto River (AP), 92.8% of the examined hosts fish had their gills parasitized by *Ichthyophthirius multifiliis* Fouquet, 1876 (Protozoa, Ciliophora), *Gussevia asota* Kritsky, Thatcher & Boeger, 1989 (Monogenoidea, Dactylogyridae) and metacercariae of *Posthodiplostomum* Dubois, 1936 (Digenea, Diplostomidae) and their intestine by adult forms of *Procamallanus* (*Spirocamallanus*) *inopinatus* Travassos, Artigas & Pereira, 1928 (Nematoda, Camallanidae) and larvae of *Contraecum* Railliet and Henry, 1912 (Nematoda, Anisakidae). High levels of infection by monogenoidea were observed

in *O. bicirrhosum*, while the highest rates of infection in *A. ocellatus* were caused by helminthes

G. asota, *P. inopinatus* and *Contracaecum* sp. (Table1).

Table 1. Parasites in *Osteoglossum bicirrhosum* from the Coari Grande River, State of Amazonas (central Amazon) and in *Astronotus ocellatus* from Preto River, State of Amapá (eastern Amazon). P: Prevalence, MI: Mean intensity, MA: Mean abundance and TNP: Total number of parasites.

| Fish species Taxa/Parasites | <i>Osteoglossum bicirrhosum</i> (N=32) | | | | <i>Astronotus ocellatus</i> (N=28) | | | |
|--|--|-------|-------|-------|------------------------------------|----------|-------|---------|
| | P (%) | MI | MA | TNP | P (%) | MI | MA | TNP |
| PROTOZOA | | | | | | | | |
| <i>Ichthyophthirius multifiliis</i> | - | - | - | - | 42.9 | 11.495.1 | 6.569 | 183.921 |
| MONOGENOIDEA | | | | | | | | |
| <i>Gonocleithrum aruanae</i> | 87.5 | 340.1 | 297.6 | 9.523 | - | - | - | - |
| <i>Gussevia asota</i> | - | - | - | - | 39.3 | 76.9 | 46.7 | 1.307 |
| NEMATODA | | | | | | | | |
| <i>Procamallanus</i> (S.) | - | - | - | - | 39.3 | 3.7 | 2.3 | 63 |
| <i>Contracaecum</i> sp. (larvae) | - | - | - | - | 25.0 | 2.4 | 0.6 | 17 |
| DIGENEA | | | | | | | | |
| <i>Posthodiplostomum</i> sp. (metacercariae) | - | - | - | - | 21.4 | 5.3 | 1.1 | 32.0 |

With regard the species richness in the community, there was predominance of parasitized

fish for *A. ocellatus*, mainly by one and three species (Figure 1).

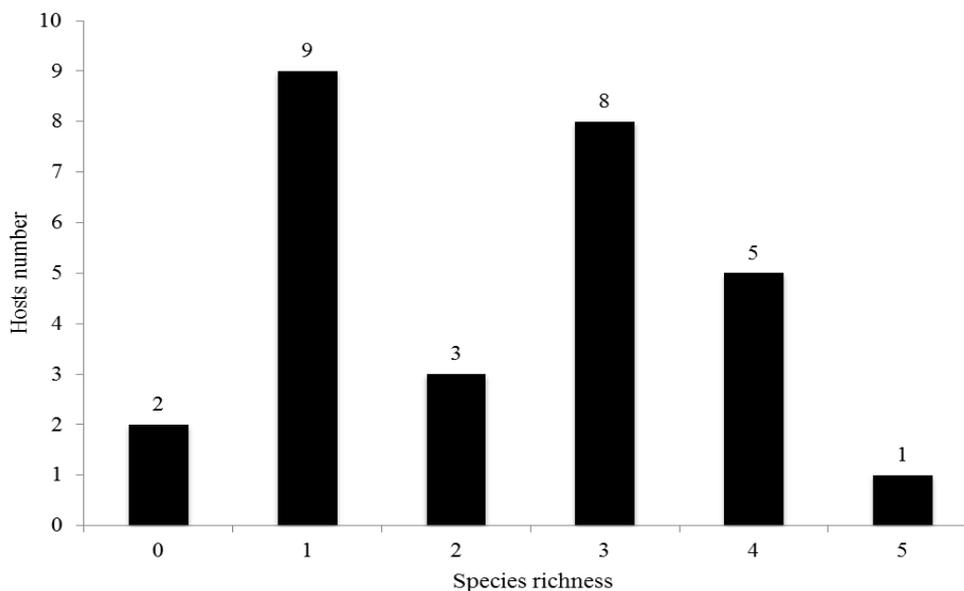


Figure 1. Species richness of parasites in *Astronotus ocellatus* from Preto River, State of Amapá, eastern Amazon.

There was no correlation of *G. aruanae* intensity with the body weight ($r_s = -0.178$; $p = 0.360$), total length ($r_s = -0.290$; $p = 0.134$) or Kn ($r_s = -0.187$; $p = 0.337$) from *O. bicirrhosum* (Figure 2). Similarly,

there was no correlation of helminthes intensity with the body weight ($r_s = 0.037$; $p = 0.854$) and the total length ($r_s = 0.046$; $p = 0.820$) from *A. ocellatus*

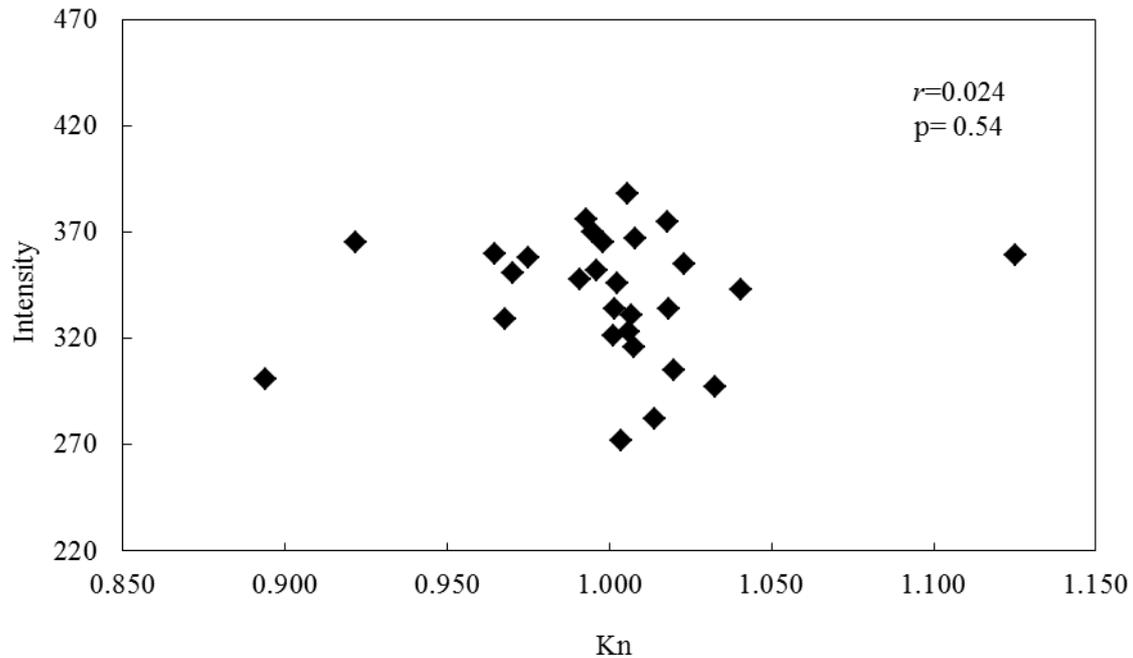


Figure 2. Correlation between the intensity of monogenoideans *Gonocleithrum aruanae* and the Kn in *Osteoglossum bicirrhosum* (N=28) from the Coari Grande River, State of Amazonas, central Amazon, Brazil.

The Kn of *A. ocellatus* showed negative correlation with the intensity of helminthes (Figure

3), which indicates that the hosts body condition was affected by these parasites in.

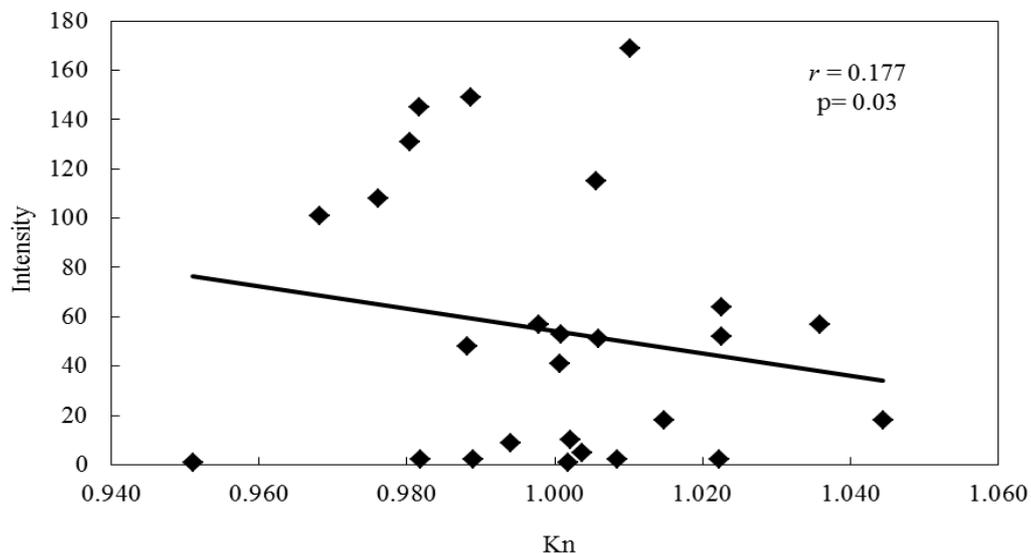


Figure 3. Correlation between the intensity of helminthes (*Gussevia asota*, *Posthodiplostomum metacercariae*, *Procamallanus inopinatus* and *Contracaecum* larvae) and the Kn in *Astronotus ocellatus* (N= 26) from Preto River, State of Amapá, eastern Amazon, Brazil.

DISCUSSION

Helminthes were the main components of the parasitic fauna of *A. ocellatus* from the Preto River in eastern Amazon, and *O. bicirrhosum* from the Coari Grande River in central Amazon, both

omnivore fish species. However, parasite fauna of *A. ocellatus* accounted on one Protist, one Monogenoidea, one Digenea and two Nematoda, while *O. bicirrhosum* was parasitized only by *Gonocleithrum aruanae*. Such results may be due to different life history of these hosts, as well as the

heterogeneity from environmental and hosts' biological factors.

Protozoan parasites have a wide range of life-cycles and cause diseases in freshwater fish. Among the most important ectoparasites of fish are the ciliated Protozoans; the most pathogenic of them is the *I. multifiliis*, which infects fish either from tropical or temperate regions (TAVARES-DIAS et al., 2001a; DICKERSON, 2006; EIRAS et al., 2010). In addition, it has worldwide distribution and infects any farmed or wild species, from juvenile to adults, and may cause high mortality rates in fish farms. Besides, when natural outbreaks of ichthyophthiriasis occur in wild fish populations, they can have devastating effects (RAISSY et al., 2010). In the gills of *A. ocellatus* from Preto River in State Amapá, there was high parasitism by *I. multifiliis*, similar to what occurred in *Capoeta damascina* from Armand River, Iran (RAISSY et al., 2010). However, infection levels were higher than the ones for *Oxydoras niger* from Lake Coari, in the state of Amazonas, Brazil (SILVA et al., 2011) and for *Capoeta aculeata* from the Choghakhor lagoon, Iran (RAISSY; ANSARI, 2011). In Brazil, despite the occurrence of *I. multifiliis* in some fish from natural environments, there are still no reports of outbreaks.

In the gills of arowana *O. bicirrhosum* from Coari Grande River (in State of Amazonas), only the Monogenoidea *G. aruanae* was found. However, for this same fish from Januacá Lake, also in the State of Amazonas, Kritsky; Thatcher (1983) identified other four species of monogenoideans, *G. coenoideum*, *G. cursitans*, *G. planacroideum* and *G. planacrus* besides the *G. aruanae*. In the gills of Oscar *A. ocellatus* from the Preto River, there was infection by monogenoidean *G. asota*, while for this same host in the Guandu River (State of Rio de Janeiro), there was parasitism by *G. asota* and *G. astronoti* (AZEVEDO et al., 2010).

Infections by monogenoideans *Gonocleithrum* spp. in *O. bicirrhosum* from Coari Grande River (AM) were higher than those caused by *G. asota* in *A. ocellatus* from the Preto River (AP). Although the prevalence of *G. asota* in *A. ocellatus* from this study was lower than the prevalence for this same host from the Guandu River (AZEVEDO et al., 2007), the intensity and abundance here were higher. Such differences are due to the fact that the hosts are benthopelagic fish from different environments. Since monogenoideans parasites have a direct lifecycle, they occur more frequently in lentic environments, which favor their transmission (AZEVEDO et al., 2007; GRAÇA; MACHADO, 2007) to hosts such as *A. ocellatus*,

which show a preference for this type of habitat (AZEVEDO et al., 2007). However, during the breeding period, the arowana *O. bicirrhosum* nests in mud and stones, in areas of calm water for the spawning and care of its offspring. During three months of parental care (SANTOS et al., 2006; SOARES et al., 2008), parents do not feed and therefore are more exposed to infections by parasites.

Many endohelminth parasites have complex life-cycles that involve two or more hosts, because they have indirect life-cycles. Larval stages occur in one or more intermediate hosts, followed by sexual reproduction of mature adults in the definitive host. Intermediate hosts are essential for the completion of larval development and the parasite sometimes undergoes a period of asexual multiplication within them. Paratenic hosts are additional non-obligatory hosts in the lifecycle, which larval parasites can infect but in which they do not undergo any further development before infecting the next intermediate or definitive host. Intermediate and paratenic hosts are often affected by the presence of these parasites, becoming more susceptible to predators, which are the definitive hosts, and thus allowing the completion of parasite lifecycle (THATCHER, 2006; BULLARD; OVERSTREET, 2008; POULIN; LEUNG, 2011).

Fish can be intermediate, paratenic or definitive hosts from nematode species (THATCHER, 2006; TAKEMOTO et al., 2009; EIRAS et al., 2010). In this study, it was observed the presence of *Contracaecum* sp. larvae and *P. inopinatus* adults in *A. ocellatus*, similarly to what was reported for *Metynniss lippincottianus* from the Paraná River (MOREIRA et al., 2009). Nematodes of the genus *Contracaecum* are parasites that in the adult stage are preferably found in fish-eating birds, which are their definitive hosts. However, fish can act as intermediate or paratenic hosts (CHOUDHURY; COLE, 2008; MOREIRA et al., 2009). Fish acting as paratenic hosts get the parasites by predated smaller fish, which in turn become infected through the ingestion of infected copepods, mollusks, gastropods, coelenterates and/or ctenophores or even larvae of *Contracaecum* sp. on its free-living stage (MADI; SILVA, 2005; CHOUDHURY; COLE, 2008). Therefore, *Contracaecum* spp. at larval stage is often found in intermediate fish host (CHOUDHURY; COLE, 2008). Chironomids are intermediate hosts of *P. (S) inopinatus* (MOREIRA et al., 2009), which have been listed as the most frequent nematode species parasitizing Brazilian freshwater fish (EIRAS et al., 2010), because it has no parasitic specificity.

For *A. ocellatus* from Preto River (in the eastern Amazon), the rates of infection by *Contracaecum* sp. larvae were similar to those of *P. inopinatus*. However, these rates of parasitism by *Contracaecum* sp. larvae were lower than those described for *A. ocellatus* from Guandu River in State of Rio de Janeiro (AZEVEDO et al., 2007). However, the rates of infection by *P. inopinatus* in *A. ocellatus* in this study were similar to those reported for *M. lippincottianus* (prevalence= 43.2% and mean intensity= 2.3) from the Paraná River basin, Brazil (MOREIRA et al., 2009). In contrast, they were lower (prevalence= 60.0% and mean intensity= 3.8) than the rates of infection described by VITAL et al. (2011) for the carnivorous fish *P. nattereri* from Lake Piranha in the State of Amazonas. These differences may be due to the different host species, environment and distinct lifestyles.

The main pathogenic effects of digeneans infections in fish hosts occur from metacercariae. In general, metacercariae tends to be less host-specific than adult digeneans and can affect fish growth and survival, as well as being a source of infection to definitive hosts and other piscivorous vertebrates. Many metacercariae enter the host fish by active invasion of the cercariae, and the transmission success depends on the cercariae behavior (THATCHER, 2006; BULLARD; OVERSTREET, 2008). Thus, distribution of metacercariae within the host can be affected by parasite behavior as well as by host behavior and habitat (THATCHER, 2006; BULLARD; OVERSTREET, 2008; TAVARES-DIAS et al., 2011). Therefore, the metacercariae can infect various fish organs, including the gills, causing moderate to severe hyperplasia of the primary gill lamellae, forming cysts surrounded with multiple cellular reactions, including mononuclear inflammatory cells (BULLARD; OVERSTREET, 2008; EISSA et al., 2011). In addition, a massive and widespread infection by these metacercariae, particularly in little fish, can cause intensive inflammatory response and mortality (BULLARD; OVERSTREET, 2008; EIRAS et al., 2010).

For this study, the levels of infection by *Posthodiplostomum* sp. metacercariae in the gills of *A. ocellatus* from Preto River were lower than those reported by TAVARES-DIAS et al. (2011) for this same host from Pracuúba Lake also in the state of Amapá. In Brazil, only metacercariae of *Posthodiplostomum macrocotyle* have been identified in the hosts *Mugil liza*, *Geophagus brasiliensis* and *Trachelyopterus striatulus* from Guandu River (AZEVEDO et al., 2010). High prevalence and intensity of

Posthodiplostomum sp. metacercariae indicates abundance of fish-eating bird in the area, which are definitive hosts, besides favorable conditions for the first intermediate host, a snail, the most common cause of the infections on the gills of *A. ocellatus* (TAVARES-DIAS et al., 2011). Furthermore, these results corroborate the fact that the fish lifestyle has great importance on the levels of infection by digeneans species.

The host-parasite relationship has been a useful tool for understanding the ecological relationships between parasites and their hosts (SILVA et al., 2011; VITAL et al., 2011; GUIDELLI et al., 2011). The body size (weight and length) and the hosts' condition factor can be used for this purpose. In this study, there was no correlation between the intensity of parasites and size of any of the host species. Similar results have also been described by Azevedo et al. (2007) for *A. ocellatus*. In this study, the lack of correlation between the body size and parasitic intensity might be due to its behavior and physiology. In contrast, the total length was negatively correlated with the abundance of digenean *Saccocoelioides nanii* and the prevalence of the metacestode for *Prochilodus argenteus* (MONTEIRO et al., 2009). POULIN; LEUNG (2011) stated that any relationship between host fish body size and the intensity of helminth parasites using it as intermediate host should be negative, because large hosts are unlikely to serve as prey. Therefore, the host fish size is not one of the factors promoting local diversification of helminth parasite species.

The condition factor is a good indicator of fish well-being and can be used to evaluate the effects of parasitism on the hosts' health (SILVA et al., 2011; GUIDELLI et al., 2011). However, the relative condition factor is indicator of health that also reflects recent nutritional conditions (GUIDELLI et al., 2011) and physiological status. In *A. ocellatus* of this study, the Kn was negatively influenced by the intensity of helminthes *G. asota*, *Posthodiplostomum* sp. (metacercarie), *P. inopinatus* and *Contracaecum* sp. (larvae), which indicates that these parasites were pathogenic to the host. However, the intensity of *G. aruanae* did not influence the Kn in *O. bicirrhosum*. In contrast, a positive correlation between the intensity of monogenoideans and Kn was reported for *O. niger* from Coari Lake (SILVA et al., 2011) and *Pygocentrus nattereri* from Piranha Lake (VITAL et al., 2011), both in the state of Amazonas. However, hosts are not affected by parasites when there is balance in the parasite–host relationship. Negative effects of endoparasites on the hosts Kn

are more prominent in infections by larvae (GUIDELLI et al., 2011), as occurred in *A. ocellatus* of this study. Furthermore, overall the response of hosts to parasites varies according to type and stress intensity caused by parasites, as well as their life-cycle and time of exposure.

In conclusion, our results showed that the helminth parasites had negative effect on body conditions of *A. ocellatus* from Preto River in State of Amapá. Given the poor fauna of endoparasites of *O. bicirrhosum* is somewhat intriguing, thus needing further studies. This study lists Preto River as a new

locality for *Contracaecum* sp., *Posthodiplostomum* sp. and *P. inopinatus*.

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RESUMO: O presente estudo avaliou a fauna parasitária de *Osteoglossum bicirrhosum* da Amazônia central (Brasil) e *Astronotus ocellatus* da Amazônia oriental (Brasil) de ambiente natural, e também a relação parasito-hospedeiro. 87,5% dos *O. bicirrhosum* estavam parasitados por *Gonocleithrum aruanae* (Monogeneoidea) e *A. ocellatus* (92,8%) estavam parasitados por *Ichthyophthirius multifiliis* (Ciliophora), *Gussevía asota* (Monogeneoidea), metacercárias de *Posthodiplostomum* sp. (Diplostomidae), adultos de *Procamallanus inopinatus* e larvas de *Contracaecum* sp. (Anisakidae). Em *O. bicirrhosum* os níveis de infecção por *Gonocleithrum aruanae* foram elevados, enquanto em *A. ocellatus* os maiores níveis de infecção foram causados por helmintos ectoparasitos como *G. asota*, e endoparasitos como *Posthodiplostomum* sp., *Procamallanus inopinatus* e *Contracaecum* sp. Em *A. ocellatus* houve significativa ($p > 0,05$) correlação negativa entre a intensidade de helmintos e fator de condição relativa (Kn), indicando os parasitos foram patogênicos para os hospedeiros. A fauna parasitária de *O. bicirrhosum* foi reduzida, enquanto a de *A. ocellatus* foi constituída por espécies de helmintos, incluindo formas larvais desses parasitas. Tais diferenças na fauna de parasitos de ambos os peixes onívoros pode ser devido a sua história de vida, bem como a fatores ambientais e biológicos.

PALAVRAS-CHAVE: Fator de condição. Helmintos. Parasitos. Peixes de água doce.

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